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OF

BACTERIOLOGY

VOLUME XVII

BALTIMORE, MD.
1929

Medical
Wm. B. Wilkins

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THIRTIETH ANNUAL MEETING OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

RICHMOND, VIRGINIA, DECEMBER 27, 28, 29, 1928

Headquarters, Hotel Jefferson

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GENERAL BACTERIOLOGY

1. Dissociation of Acido-proteolytic Bacteria by the Climbing Culture Method. PROF. DR. CONSTANTINO GORINI, R. Scuola Superiore D'Agricoltura, Milan, Italy.

The acido-proteolytic bacteria, discovered by me in 1892–1894[1] attack both carbohydrates and proteins simultaneously. They are able, therefore, to peptonize even in an acid medium.

[1] Atti dei Laboratori Sanità Publica al Minist. Interni, Roma, 1892. Revista d'Igiene e Sanità Publica, Roma, 1893, **4**, 549; Hyg. Rundschau, 1893, **3**, 381; Giornale della reale Soc. ital. d'Igiene, 1894, **16**, No. 4.

The climbing culture method, proposed and described by me in 1903[2] consists in the inoculation of the condensation water of a sloped tube of agar with bacteria which have a tendency to grow as a spreading film on the surface of the medium (*B. proteus, B. typhi, B. tetani,* etc.). By this method these bacteria may be easily isolated from diverse materials.

Among the acido-proteolytic bacteria described by me in 1904[3] is *Bacillus acidificans-presamigenes-casei.* This is a spore-bearing bacillus similar in form to *B. subtilis.* This organism exhibits physiological mutations, having some cells which are predominantly proteolytic and others which are predominantly acidifiers.[4] Two types of colonies corresponding to these two types of processes are found on gelatin plates. A granular type has been called the unstable S type, and a filamentous type has been called the stable R type.[5]

Having observed that the granular type is much more motile than the filamentous type, I have applied the climbing culture method to cultures of *B. acidificans-presamigenes-casei.* In this way I have obtained the isolation of the granular type, making a series of transfers from the top of the spreading edge of the growth.

From this I conclude that my climbing-culture method may be employed not only for the isolation of motile or spreading species, but also for the microbic dissociation of species whose cells have a motile phase in their evolution.

2. The Rate of Growth of a Colony of Bacteria. L. A. ROGERS AND GEO. R. GREENBANK, Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

Growth in a tube filled with nutrient broth does not progress uniformly but by alternate periods of rapid and slow growth.

3. Physiological Difference Between Young and Old Bacterial Cells. C. N. STARK AND PAULINE STARK, Cornell University, Ithaca, N. Y.

Differences between young and old bacterial cells have been established through studies of the relative rates of fermentation and the relative resistance to heat of cells of different ages.

[2] Rend. R. Ist. lombardo di Sci. e. Lett., 1903, **36,** 601.

[3] Rend. R. Ist. lomb. di Sci. e. Lett., 1904, **37,** 939; Centralbt. f. Bakt., II Abt., 1906, **16,** 236.

[4] Rend. R. Accad. dei Lincei, 1921, **30,** 312; J. Dairy Sci., 1922, **5,** 510.

[5] Rend. R. Accad. dei Lincei, 1928, **7,** 689; C. r. Acad. Sci., Paris, 1928, **186,** 1070.

The rate of fermentation per cell per hour was calculated by use of the formula

$$X = \frac{S \log \frac{b}{a}}{t(b - a)\ 0.434},$$

where X = amount of acid formed by one cell in one hour
a = number of bacteria at beginning
b = number of bacteria at end
t = time in hours
S = acid formed in time t

It was found that the average rate of fermentation of young cells of *Bacterium coli* was 45.5×10^{-10} milligram acid per cell per hour, while the average rate for old cells in a similar medium and under the same conditions was 9.9×10^{-10} milligram acid per cell per hour.

With respect to heat resistance, it has been shown that the young cells of *Streptococcus fecalis* and 2 types of capsule forming organisms are much more sensitive to heat than are old cells of the same organisms.

4. Bacterial Calorimetry. S. Bayne-Jones and Henrietta S. Rhees, Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

By means of simultaneous observations of the heat production by bacteria and enumeration of the organisms in the culture it is possible to correlate growth curves with curves of the liberation of heat. A combination of such observations, with those of the morphological changes and life-phases of bacteria, yields some information as to the metabolic activities of a culture at various periods. By the use of Buchanan's formula, the rate of heat production by a single bacterial cell can be calculated, and the observed values agree closely with those derived by this calculation.

Changes in the rate of heat production are related to changes in the growth rate of bacteria.

Young bacterial cells produce more heat per cell than older ones.

After about the fifth hour of growth, the production of heat reaches a low level and remains constant, suggesting the existence of a basal metabolic rate during that period.

5. Remarks on Two Modes of Cell Division in Bacteria. GEORGES KNAYSI, Department of Dairy Industry, Cornell University, Ithaca, N. Y.

In the dividing cell of *Mycobacterium tuberculosis*, a sharp clear zone of division, perpendicular to the long axis of the cell, is observed first. The stainable constituents of the protoplasm are drawn back into two dense masses which usually persist at each pole of the daughter cells. The portions of the membrane of the mother cell which limit the clear zone remain for a while until new membranes limit the zone when it withers away. No constriction of the cell at the zone of division was observed during the process.

In the dividing cell of *Proteus vulgaris*, one of the surface granules of the cell membrane grows in and increases in size until it reaches the other side or, more often, two such granules at opposite sides may grow and extend until they meet. At the same time, or sometimes immediately afterwards, the cell becomes more and more constricted at the zone of division. It is probable that the new partition is split by furrowing to form the closing membranes. In the majority of cases the cells remain connected by means of plasmodesma, the chemical nature of which has not yet been ascertained.

It seems, therefore, probable, from the above descriptions and from a close study of the structure of the bacterial cell, that surface tension plays a secondary, if any, rôle in the process of cell division.

6. The Value of Vegetable Extracts in Culture Media. LUTHER THOMPSON, Mayo Clinic, Rochester, Minn.

Extracts were made of potato, carrot, radish, spinach and of beef heart, the latter for comparison. One hundred and fifty grams of grated or ground material were added to 200 cc. of distilled water. The mixture was shaken for ten minutes and then strained and sterilized by filtration.

Potato extract was found to be the most satisfactory of those tried. It gives a total nitrogen of about 1.2 mgm. per cubic centimeter and approximately 0.2 per cent reducing sugars. Amounts as little as 0.01 cc. when added to 6 to 7 cc. of nutrient broth will give growth with many strains of streptococci which will not develop in the broth alone while 0.2 cc. is enough to secure a vigorous growth with nearly all strains encountered.

The growth accelerating factor is not destroyed by heat except when heating causes a precipitate, but is destroyed by the action of certain bacteria. It is thought to be a protein which is easily available to

bacteria, and not a vitamine or growth accessory factor, since similar results may be obtained with the products of bacterial decomposition of peptone.

Potato extract is useful as a substitute for blood in growing streptococci. When added to meat infusion media, it allows the growth of *Hemophilus influenzae*. It may also be used in small amounts as an enriching substance in sugar fermentation tubes instead of serum or meat infusion.

7. *The Radiation of Ultraviolet Light—Mitogenetic Rays, So-Called—By Bacteria and Higher Plants.* RALPH R. MELLON, N. AND E. VON RASHEVSKY, Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa.

Within recent years Gurvitch has brought forward rather interesting experiments indicating that growing plant tissues emit a short wave length ultraviolet light capable of stimulating cell division and growth enormously. He therefore spoke of the rays as mitogenetic from the large number of mitoses observed in the plants subjected to their action. He worked chiefly with the rootlets of growing onions but also with other plants and embryonic animal tissues. Positive results were so frequent as to lead him to conclude that the phenomenon might be a general one.

The effect would seem to be one of radiation and not electron emanation. This belief is supported by the fact that quartz does not appear to bar its passage, while glass does. Moreover, the radiant energy is capable of diffraction and is absorbed by thick layers of air and by gelatin. Inasmuch as his attempts to photograph the light (?) were failures, he inferred that its wave length lay between 1900 and 2000 A.U. He believed that the gelatin of the photographic plate absorbed the light.

Certain anomalous experiences of our own in connection with the division and differentiation of bacterial cells gave impetus for a photographic demonstration of this phenomenon. If successful in this, it was our purpose to extend the observations to the bacteria. In both of these considerations we have been successful.

We have circumvented the photographic difficulty encountered by Gurvitch by sensitizing the ordinary photographic plate with machine oil, which is known to fluoresce in the presence of ultraviolet light, thus giving a visible light to which the plate is, of course, sensitive. It was found that *B. coli*, as well as growing onions, when exposed to the

sensitized plates for three to five days affected them very appreciably. It is not known as yet how universal this phenomenon is among the bacteria, but our observations on this point are being somewhat extended.

8. Certain Biochemical Reactions Produced by the Streptococci. G. J. HUCKER, New York Agricultural Experiment Station, Geneva, N. Y.

A study was made of a large number of representative strains of streptococci to find constant and convenient characters by which the species of the genus may be differentiated. Only authentic and previously named strains of streptococci were investigated and no effort was made to make a floral study of any particular natural habitat of this group.

The streptococci can be divided into two groups on the basis of the type of lactic acid produced. The low acid producers, which generally prefer levulose to glucose, attack the pentoses, form relatively large amounts of volatile acid, and many times produce carbon dioxide, all form levo-lactic acid. The more common types of streptococci all produce dextro-lactic acid.

Many of the strains were found to attack the nitrogenous constituents in milk and produce an increasing amount of water-soluble nitrogen if the culture was allowed to incubate over a long period of time in the presence of calcium carbonate. Certain of the strains studied, particularly *Streptococcus lactis* Löhnis and related types, produced less than 10 per cent of the total acidity as volatile acid, while *S. kefir* Migula, the aroma types, and certain hemolytic strains produced as high as 40 per cent volatile acid. The relative amounts of non-volatile and volatile acid formed remained approximately constant regardless of the total amount of acid produced.

The non-hemolytic strains all produce acetic acid and a volatile acid with a higher distilling constant than acetic, while the hemolytic types form, in addition to acetic acid, a volatile acid with a lower distilling constant than that found for acetic acid. The distilling constants obtained from the hemolytic strains indicate that varying amounts of formic acid are present.

Constant results were obtained in qualitative acid production from sugars if the conditions of growth were controlled, and it was concluded that fermentations of sugars and other carbon sources were valuable as routine methods in separating the species of streptococci.

The strains producing levo-lactic acid were found to hydrolyze the lactose much faster than it was converted into acid, while the remaining types produced acid as rapidly as the lactose was hydrolyzed.

There is a great need for a usable classification of the streptococci, one which is based upon constant and easily determined characters and there is evidence that the biochemical reactions produced by the streptococci studied may be used as a basis for differentiating the species in this genus.

More detailed information may be found in New York State Agricultural Experiment Station Technical Bulletin No. 143.

9. Further Studies on Certain Aciduric Organisms of Dental and Salivary Origin. Toshiki Morishita, Laboratory of General Bacteriology, Yale University, New Haven, Conn.

In a previous paper the author briefly discussed one of several types of apparently distinct aciduric organisms found in dental caries and in the saliva of persons having carious teeth. This type may briefly be described as follows. The members of this group grow at 12 to 14°C., coagulate milk, and ferment mannose, trehalose, melezitose, mannitol, sorbitol, and salicin, but do not attack sucrose and raffinose. They are agglutinated only by homologous sera.

The present report deals with a second distinct group or type from the same source. This organism is Gram-positive, occurs in rather slender rods, singly or in chains, and tends to form palisade-like groupings. All agar colonies are solid. The different strains do not as a rule coagulate milk; freshly isolated strains may rarely curdle milk but later lose this property. Raffinose and sucrose are fermented. There is no action on trehalose, melezitose, dextrin, sorbitol, mannitol and salicin. The fermentation of mannose is irregular. All mannose non-fermenters are able to grow at 18°C., while many of the mannose-fermenters fail to develop at this temperature. Neither of these 2 sub-types grew at 12 to 14°C. Both produce a large amount of acid in sugar broths and are able to proliferate or remain viable in very acid media. Growth is markedly inhibited by an alkaline reaction. There are at least 2 immunological groups, as determined by means of the agglutination tests.

Group II may be distinguished from *L. acidophilus* (Moro) as follows: (1) While some strains of this group cross-agglutinate in the lower dilutions of heterologous sera, no cross agglutination is observed in the higher dilutions. The presence of these specific and non-specific agglutinins can be demonstrated by agglutinin absorption tests;

(2) by their action in milk; (3) by fermentation of mannose, trehalose, dextrin and salicin; (4) by their relative sensitiveness to chemical reactions of media; (5) by growth at lower temperatures. Differences in morphological and cultural characteristics were also found to be of value in differentiation.

Out of a total of 105 strains of aciduric organisms isolated from dental caries and from saliva, 30 strains may be placed, because of their biological reactions, in the dual group reported in this paper. When considered from an immunological viewpoint, this number is increased to 39.

Forty-two out of 105 strains fall into the group reported in the author's previous paper. Scattered representatives of a few other groups are also found among the remaining strains.

Not more than 7 of the 105 isolations bear a close resemblance to *L. acidophilus* (Moro). The more infrequent types are perhaps more or less transient, and from the standpoint of possible relationship to dental caries are of little or no real significance.

10. A Contribution to the Classification of Microörganisms Treating the Orders Eubacteriales and Actinomycetales. ERNST PRIBRAM, Loyola University School of Medicine, Chicago, Ill.

I. Discussion of general rules for a systematic classification:

1. Characteristics of the higher ranks should contain only the common characteristics, i.e. those characteristics which they have in common with *all* subordinated groups (orders, families, tribes), not the characteristics of some of them. Example: Class Schizomycetes: "Undifferentiated unicellular microörganisms which multiply by cell fission." It is not necessary to add: "some of them are spore formers." All spore-forming bacilli are included, because they all multiply by fission also.

2. Characteristics of subordinated groups include of course all characteristics of the higher rank to which they belong and the characteristics of this higher rank ought not be repeated.

3. The fundamental principle of characteristics, as proliferation, metabolism, morphology, biological properties should be uniform for all the coördinated members of one group (class, order, family, tribe).—It is accepted as a general botanical rule, that the form of reproduction is of primary importance as far as characteristics are concerned. The general metabolic properties as for instance direct assimilation of elements might be superior to morphological characteristics and these latter again superior to biological properties such as fermentation, pigment production and pathogenicity or toxin production.

4. In a classification one should consider, that there are transitional forms between all species, genera, families, orders and even classes. Example: Spirochetales between Protozoa and Schizomycetes, Actinomycetales between Schizomycetes and Eumycetes. There are therefore characteristics of both groups present in one transitional form. It would be advisable to qualify these groups as *connecting links* and others as *standard groups*. Connecting links have usually species, which vary with greater ease than standard forms (transitional forms). The variation of the connecting links may be due to one or more properties.

5. When classifying fermenting microörganisms according to their power to ferment carbohydrates we should keep in mind that not all the carbohydrates are equivalent. Special studies will be necessary for different families. The property, to ferment lactose for instance, which can be used as one of the main characteristics of the *Escherichia* genus, does not mean simply a difference between the fermentative power of this genus and the other coördinated genera, as *Salmonella*, *Eberthella* etc., but signifies, that the genus *Escherichia* is saprophytic and prefers milk, while the other genera are either parasitic or adapted to other organic products. There are similar findings among different saccharomycetes, e. g., lactase which is a rare ferment of yeasts, has been found in those forms, which are isolated from milk (*Sacch. kefir.*).

6. The reaction with the Gram stain should be widely recognized as a principle of classification, because it very often runs parallel with other important properties as for instance spore formation or anaerobic growth. The Gram technique is not difficult and generally yields good results.

II. In accordance with these principles an attempted classification on the basis of the classification and nomenclature of the Society of American Bacteriologists will be discussed and submitted to this Society. A few names used for subdivisions and some connecting links which have not been approved by the Committee of the Society may be considered as tentative and further discussion on them is invited.

The following families and genera will be discussed with special regard to their position in a systematic classification:

Family Nitrobacteriaceae (completely).
Family Spirillaceae with regard to connecting links.
Family Bacteriaceae. Discussion and classification of all tribes and genera.
Family Bacilleae, remarks on some species.
Family Actinomycetaceae. All tribes and genera.
Family Mycobacteriaceae. All tribes and genera.

11. A Convenient Case for Sterilizing and Storing Micropipettes. WILLIAM H. WRIGHT, University of Wisconsin, Madison, Wisconsin.

The ease with which the delicate tips of micropipettes may be broken during handling and sterilization makes necessary a safe and convenient method of holding them until they are used. The older vertical types of cases for handling micropipettes have the disadvantage that the tips of the pipettes are always upward, and in placing them in such cases as well as when they are taken out they are very liable to be broken or contaminated.

A case of new design has been perfected, and is arranged so that any pipette can be removed at will without molesting the others or running any risk of breakage. In this type of case the pipettes lie parallel to each other in a horizontal row, each one being held in place by an individual spring. Any pipette may be removed at will by one movement of the hand.

12. The Growing of Pure Cultures from Single Cells of Non-Spore Forming Bacteria. WILLIAM H. WRIGHT AND HISASHI NAKAJIMA, University of Wisconsin, Madison, Wisconsin.

A modified cultural technique is described in which the accessory to the Chambers' apparatus, as described by Wright and McCoy, is used to good advantage for both isolating and culturing successfully single cells of *E. coli* and *Pneumococcus*, Type I.

The use of test tube cultures has been abandoned in favor of hanging-drop cultures in special hanging-drop slides with very deep wells. A modified technique is used, in which a small droplet containing a single cell is deposited near the edge of a larger drop of sterile liquid medium on a sterile cover glass over the moist chamber on the stage of the microscope.

By manipulating the tip of a sterile micropipette so that the side of the larger drop near the small droplet is, for an instant only, pulled toward the smaller drop, the two drops are caused to become confluent, and the single cell may be seen to float away into the larger drop.

This technique is more sure than any yet devised and at no time in the procedure is the isolated single cell out of sight of the observer. In the previous method where the tip of the micropipette was broken off in a tube of sterile liquid medium there was always the uncertainty of what had become of the single cell and also a slight chance for air contamination.

During the isolation of over 600 single cell cultures of *E. coli* and

Pneumococcus, Type I, with this improved technique, not a single contamination has occurred. In every case where the single cells transferred to the larger drops did not grow the drops remained sterile for several days until the cultures were discontinued.

Cultures of single cells of non-spore forming bacteria appear to be affected by the following conditions.

1. The relation of the amount of culture medium, (size of culture drop), to the volume of the single cell. 2. Composition of the culture solution from a qualitative point of view. 3. Density of the medium used for the drop cultures. 4. The age of the culture from which the single cells are taken for the drop cultures. Cells give more cultures which grow, when they are removed from cultures in the logarithmic stage of development. This has to be determined by making preliminary trials and counts of the number of cells developing in the medium used and under the conditions used for the drop cultures.

Using this procedure 46 per cent of the single cell cultures of *E. coli* and about 20 per cent of the single cell cultures of *Pneumococcus*, Type I, have been grown.

13. The Eijkman Fermentation Test as an Aid in the Detection of Fecal Organisms in Water. LABAN W. LEITER.[1] Presented by WILLIAM W. FORD, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

In 1904 Eijkman reported that *Bacillus coli* from man and warm-blooded animals has the ability to ferment glucose broth, with acid and gas production, when incubated at 46°C. and that the contamination of water by fecal organisms can be detected by this method of examination. It has been found, in applying this test to various waters in Baltimore, that it is selective for *Bacillus coli* and that other organisms in water are inhibited or destroyed. It is complete in twenty-four hours. It yields members of the aerogenes-cloacae group infrequently, in marked contrast to the results of Standard Methods. The great majority of the strains of *Bacillus coli* from man and warm-blooded animals are able to ferment glucose broth when grown at 46°C. and the test correlates well with indol production, non-utilization of Koser's sodium citrate and uric acid media. Many strains of bacteria giving the reactions of *Bacillus coli* from feces of cold-blooded animals ferment glucose

[1] Deceased.

broth at 37°C. but fail to grow in glucose broth at 46°C., fail to produce indol, and utilize sodium citrate and the nitrogen in uric acid medium. The Eijkman test may prove to be superior to Standard Methods in the examination of water, in that it eliminates many strains of organisms from cold-blooded animals which react like *Bacillus coli*, but are probably of no significance when found in water. Further studies of the Eijkman reaction are needed.

14. Hastening Gas Appearance in Presumptive Coli Tests. JEAN BROADHURST, Teachers College, Columbia University, New York City.

Gas formation may be seen nine to sixteen hours earlier in swimming pool water, polluted well water, etc. by centrifuging the water, and inoculating the usual lactose fermentation tubes with the bottom cubic centimeter in the centrifuged tube. Less difference was found with small samples of water, 10 cc.; with 50 cc., however, the differences were quite consistent, and occasionally, were as great as twenty-four hours in favor of the centrifuged tubes, where gas sometimes appeared in eight hours. Centrifuging was carried on for one hour, full speed, International Equipment Co., centrifuge Size 1, Type C.

15. The Favorable Rôle of Cysteine in the Cultivation of Anaerobic Organisms. GEORGE VALLEY, Laboratory of General Bacteriology, Yale University New Haven, Conn.

In their studies concerning the relationship of *B. sporogenes* (*Cl. sporogenes*) to oxygen, Quastel and Stephenson, employing casein digest broth, found that cysteine hydrochloride induced growth of *C. sporogenes* and *Cl. tetanomorphus* in an open tube. In the experiments described here it was deemed of interest to ascertain if other anaerobes would respond similarly. Nutrient broth enriched with cysteine-hydrochloride (0.1 to 0.2 per cent) yielded growth in an open culture tube with the following anaerobes: *Cl. tyrosinogenes*, *Cl. sporogenes*, *Cl. tertium*, *Cl. flabelliferum*, *Cl. chauvei*, *Cl. oedematiens*, *Cl. tetanoides*, *Cl. botulinum* (4 strains), *Cl. welchii* (2 strains), *Cl. tetani* (2 strains), *Cl. histolyticum*, *Cl. sphenoides*, *Cl. bifermentans*, and *Cl. centrosporogenes*. Growth was not obtained with *Cl. putrificum* in an open culture tube; when, however, a small inverted tube (Durham's fermentation tube) was enclosed, growth appeared in the inner tube in from twenty-four to thirty-six hours. The cysteine broth was also employed in carbo-

hydrate fermentation studies with the above-mentioned anaerobes. Results concordant with those of previous investigators were obtained showing that the fermentative and other properties of an organism grown with the aid of cysteine remained unaltered, no matter how luxuriant the growth might be. It is necessary that the cysteine broth be freshly made in order to obtain the desired results.

Methylene blue in high dilution (1:100,000) was completely reduced when added to 5 or 10 cc. of cysteine broth. Cysteine-hydrochloride in the broth medium establishes and maintains a reduced condition sufficient to initiate growth of anaerobic bacteria in an open tube. In an agar medium containing cysteine-hydrochloride (0.15 to 0.2 per cent) reducing conditions were maintained for a considerable length of time, as was shown by methylene blue decolorization. In the plain or glucose agar the indicator was immediately oxidized upon autoclaving, as was shown by the blue color formed throughout the column of agar in the tube. There was, however, a slight reduction at the bottom of the agar column after standing for eighteen hours at room temperature. Evidently auto-oxidation-reduction changes in the medium brought about this subsequent reduction. With cysteine agar the penetration of oxygen was slow, being quite effectively prevented by the reducing agent present. In eighteen hours a blue layer 8 mm. deep developed at the surface; after two days' exposure to ordinary air the blue color was restored to the depth of 20 mm. On liquefying the agar for plating, the blue color was discharged.

Open air incubation with cysteine agar did not yield surface growth on slants excepting with *Cl. tertium*, which showed a small amount of development. There was, however, growth in the condensation water, and also behind the agar, between the glass and the medium. No growth occurred on plates incubated aerobically, the plate pouring manipulation permitting oxygen to penetrate sufficiently to restore the blue color. However, in anaerobic jars, evacuated, refilled 3 times with CO_2, and finally sealed under reduced pressure (25 to 30 mm. of Hg), colony growth was luxuriant with *Cl. tetani* on cysteine-containing agar plates. On the other hand, meager development was obtained on ordinary, cysteine-free glucose agar, and none on plain agar held in the same jars during the eighteen-hour incubation period.

It appears evident that cysteine serves as an excellent reducing agent in the medium and thus establishes favorable conditions for the development of anaerobic organisms.

16. A Modification of McLeod's Anaerobic Plate. P. A. TETRAULT AND ELIZABETH MCCOY, University of Wisconsin, Madison, Wis.

A simple and yet efficient anaerobic plate may be made as follows: Two sterile Petri plates of exactly the same size are used. Into one is poured an agar, gelatin, or silica gel medium inoculated with the organism to be isolated. This is allowed to solidify. Into the second Petri plate, pyrogallic acid and sodium or potassium hydroxide are placed. The part of the first Petri plate containing the solidified medium is inverted over the half containing the pyrogallic acid-hydroxide mixture. The two halves are sealed together by means of heavy gummed paper tape. The tape is moistened and wrapped around the plate three or four times. The seal is perfect if the Petri dishes are well chosen.

The amount of pyrogallic acid and hydroxide used varies with conditions under which the organisms are incubated. For a twenty-four to forty-eight hour incubation the maximum amount of pyrogallic acid-hydroxide mixture may be used. Using van Riemsdijk's proportions, 10 cc. of 20 per cent NaOH is first placed in the plate and to this is added 3 cc. of 44 per cent pyrogallic acid. This is sufficient to absorb the oxygen from 400 cc. of air, twice the amount needed for the volume of the two Petri dishes. For the longer incubation, from five to twenty-five days, or at high temperature like 55°C. a weaker mixture must be used as the hydroxide functions as a dehydrating agent and dries the agar.

The advantages of the method are: simplicity, no special apparatus needed, large surface of pyrogallic mixture exposed, process of fishing out colonies simplified, development of colonies may be watched.

The disadvantages are: pyrogallic mixture may be splashed onto the agar, perfect seal may not be obtained if plates are not carefully selected.

17. Quantitative Aspects of the Metabolism of Anaerobes. IV. The Nature of the Volatile Acid Produced by C. histolyticum. W. S. STURGES, L. B. PARSONS AND E. T. DRAKE, Laboratory of the Cudahy Packing Company, Omaha, Nebraska.

Three strains, carefully identified as pure cultures of *C. histolyticum*, were grown on a variety of sugar-free media. Fifty-nine Duclaux volatile acid determinations are presented. They conclusively demonstrate that this species differs from all anaerobes previously studied by producing from protein a single volatile acid instead of mixtures of

volatile acids. This one pure volatile acid is produced regardless of protein substrate or age of culture.

18. Strain Variations in C. bifermentans. W. S. STURGES AND E. T. DRAKE, Laboratory of the Cudahy Packing Company, Omaha, Nebraska.

Twenty-one strains belonging to the bifermentans-centrosporogenes group (including 4 authentic strains each of *Cl. bifermentans* and *Cl. centrosporogenes*) are compared with each other and with *Cl. sporogenes* as to morphology and certain phases of cultural and biochemical behavior. Differences between *Cl. sporogenes* and the bifermentans-centrosporogenes group are greater than the differences between the individual members of the group. The data suggest that other criteria than motility or ease with which motility may be demonstrated should be used if this group is to be subdivided.

19. The Identity of Bacillus Sordellii and Clostridium oedematoides. IVAN C. HALL, University of Colorado School of Medicine, Denver, Colo.; ERWIN JUNGHERR, University of Montana, Bozeman, Mont.; AND MARION REINHARDT RYMER, Highland Hospital, Rochester, N. Y.

During a detailed study of 11 cases of human gaseous gangrene and malignant oedema in South America, Sordelli in 1923 recorded 2 strains of a new species of pathogenic obligately anaerobic bacillus, to which, because of their morphologic and cultural resemblance to *B. sporogenes* and their pathogenic resemblance to *B. oedematiens* (B. Novyi), he gave the name *"B. oedematis sporogenes."* The principal properties ascribed to these 2 strains by Sordelli were confirmed, with the exception of pathogenicity in one of them, by Hall and Scott in 1927, who proposed the binomial *"Bacillus Sordellii"* to supplant the invalid and confusing trinomial first suggested by Sordelli. The pathogenic strain was dissociated by Hall and Scott into 2 strains differing slightly in certain minor characters.

Just a few days before Hall and Scott sent their paper to press, Meleney, Humphreys and Carp described several strains of a supposedly new species of pathogenic anaerobe isolated by them from a fatal case of post-operative infection and from catgut in New York, to which, on account of the resemblance of lesions in the patient and in experimental animals to those of *B. oedematiens* (B. Novyi), and following Bergey's nomenclature, they gave the name *"Clostridium oedematoides."*

The properties ascribed to *Clostridium oedematoides* so closely resembled those of *B. Sordellii* that Hall and Scott suggested the strong possibility of their identity. Humphrey and Meleney have since affirmed it and their findings are here confirmed by detailed comparative morphologic and cultural studies of the South American strains and of the New York strains and by cross protection toxin-antitoxin tests in guinea pigs.

All of the 6 strains available are Gram-positive, obligately anaerobic, slightly motile rods forming subterminal or central clostridial spores. Coagulated albumins and gelatin are liquefied and iron brain medium is blackened. Tyrosin crystals appear in suitable media. One of the South American strains is non-pathogenic but morphologically and culturally identical with the other South American and New York strains. Five of the strains are highly pathogenic for rabbits and guinea pigs, producing lesions similar to those of *B. Novyi*, i.e. marked oedema, with slight congestion, and little or no emphysema, through the action of a true exotoxin. Antitoxic sera were produced in rabbits by the South American strains which protected against both intoxication and infection by the New York strains and vice versa.

In view of the doubtful validity of the genus "Clostridium" the attitude is taken that "*Bacillus Sordellii*" is the first valid binomial applied to this species.

20. Some Factors Influencing the Heat Resistance of Bacterial Spores. O. B. Williams, Botany Department, University of Texas, Austin, Texas.

When cultured and tested under certain standard conditions *B. subtilis* yielded spores of uniform maximum resistance. Pronounced deviations from this basic maximum resistance could be produced by changing the nutritive substrate. Spores of enhanced resistance were produced in peptone water to which had been added either salts of magnesium, phosphates, carbohydrates or certain organic acids. Cultivation in isoelectric gelatin, in various vegetable infusion media or in casein digest medium likewise resulted in the formation of very resistant spores. The temperature of incubation was found to be of some significance, the higher temperatures resulting in spores of increased resistance. The initial reaction of the medium was without influence on the spore produced.

No correlation was apparent between either extent of growth or of sporulation and the resistance of the spore produced.

Attempts to produce spores in any appreciable quantity in synthetic media prepared from highly purified chemicals were not successful.

21. Progress in Standardization of Biological Stains. H. J. CONN, New York Agricultural Experiment Station, Geneva, N. Y.

There are now 38 stains which have been put on the certification basis. There has been a noticeable improvement in the stain supply of recent years as shown by the fact that in 1923 45 per cent of the samples submitted were refused certification, in 1928 only 3 per cent. At the present time steps are under way to secure coöperation with the Federal Specifications Board in drawing up government specifications for stains in harmony with those of the Stain Commission.

AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY

1. Strain Variation in the Root Nodule Bacteria of Clover, Rhizobium trifolii. I. L. BALDWIN AND E. B. FRED, University of Wisconsin, Madison, Wis.

Eighteen strains of *Rh. trifolii* have been tested for their efficiency in aiding plant growth when in association with the host plant. Three of the strains were originally isolated from *Trifolium pratense*, two from *Tr. incarnatum*, one from *Tr. repens*, two from *Tr. suaveolens*, two from *Tr. arvense*, two from *Tr. procumbens*, and six were secured from other investigators or were commercial cultures where the original source of the isolation was unknown. Since their acquisition in this laboratory they have been carried continuously on a yeast water mannitol agar (transfers made every four to six weeks) and tested repeatedly for purity and ability to form nodules on *Trifolium* sp. One strain was secured in 1917, four in 1921, three in 1922, two in 1926, and eight in 1927. With the strains secured from other investigators or commercial cultures the absolute time since isolation from the host plant is unknown.

All tests were made in the greenhouse under controlled conditions. Plants were grown in sterilized glacial sand and watered with distilled water. Mineral nutrients without nitrogen were supplied with a modified Crone's solution. The seed was treated with hot water to kill any nodule bacteria and inoculations were made from young agar slants of the cultures. Plant cultures were always carried in triplicate with frequent controls. All cultures were tested for their effectiveness in aiding plant growth of *Trifolium pratense* and *Tr. repens* two or more times and many have been tested for their effect on other species of clover.

Considerable variation was exhibited with respect to the benefit derived by the host plant from the bacteria. Three of the strains were essentially parasitic in that the growth of the host plant was not in-

creased by the association. Two of these strains were recent isolations, 1927, from *Tr. arvense*, growing on a clover sick soil, while one was isolated in 1921 from *Tr. pratense*. A more or less gradual variation was exhibited between the poorest and the best strains. Under the conditions of these experiments more nodules were formed by the poor strains than by the good strains. The nodules, however, were smaller and were distributed over the entire root system. With the good strains fewer, but larger nodules occurred, and these were located at the upper portion of the root system.

The growth conditions determine to a certain extent the relative value of the strains. Plants grown during the winter with a short day and often little direct sunlight gave less difference between the good and poor strains than plants under conditions more favorable to photosynthesis. Under conditions of low carbohydrate metabolism the need for nitrogen is less and apparently some of the poorer strains are able to supply nitrogen compounds as rapidly as they can be utilized by the host.

2. Relation Between Electrophoresis and Certain Characteristics of the Root Nodule Bacteria. FRANZ ZUCKER, (introduced by E. B. FRED), College of Agriculture, University of Wisconsin, Madison, Wis.

In studying the electrophoretic behavior of the nodule bacteria, 71 cultures have been tested; 31 strains of *Rhizobium meliloti;* 18 of *Rhizobium trifolii;* and 22 of *Rhizobium leguminosarum.* Five day old cultures grown on mannitol yeast water agar with excess calcium carbonate were used. The velocity was determined by Falk's method as given in the Journal of Bacteriology, 15, 367–450, 1928.

The results up to the present show that a certain relationship exists between acid production and electrophoretic velocity. In general the acid formers show a higher velocity than the non-acid producers, with a dividing point at about 4.2 micra per second.

Usually the higher the velocity the "poorer" the cultures are with respect to their effect on the growth of the higher plant. The difference between these good and poor cultures is not as well defined as that between acid-forming and non-acid forming cultures. The lowest velocity was about 2.0 micra per second and the highest was about 7.2 micra per second as measured by the method of Falk. The influence of the treatment of the cultures with respect to the velocity has been studied.

3. Nitrite Production by Some Strains of Cowpea and Soybean Organisms. LEWIS T. LEONARD, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

The ability of certain strains of legume nodule bacteria to produce nitrite in media containing nitrogen compounds, has been demonstrated by some investigators; while others have failed to detect this physiological tendency. Employing principally soil extract and bean extract liquid media with various nitrates and other compounds as sources of nitrogen and sucrose, glucose, dextrin and mannitol as sources of carbohydrate, studies have been made on the production of nitrite on some members of the cowpea group of organisms and on some strains of soybean bacteria including both the high and low nitrogen-fixing types of Wright's designation. As a rule, but not without exceptions, with the cultures used in this experiment, the low nitrogen-fixing strains produced greater amounts of nitrite under given conditions than either the cowpea strains or the high nitrogen-fixing strains of the soybean. In some cases nitrite was apparently produced by the low nitrogen-fixing strains from nitrogen compounds other than nitrate, while some cultures of the other 2 strains did not cause a perceptible formation of nitrite in any of the media employed.

4. Isolation of a Bacteriolytic Principle from the Root Nodules of Red Clover. E. R. HITCHNER, Department of Agricultural Bacteriology, University of Wisconsin, Madison, Wis., and University of Maine, Orono, Maine.

An agent has been isolated from the root nodules of red clover, which produces lysis of a strain of red clover bacteria isolated from the same nodules. The lytic agent has been carried through 25 serial transfers and filtrations with its homologous organism without any loss in activity. The agent is specific for its own homologous strain and fails to produce lysis of stock strains of red clover organisms. Lysis of a twenty-four hour broth culture is complete in two days. Upon continued incubation of the lysed culture, secondary growth develops. Bacteria, isolated from such secondary growth, resist the lytic action of the agent.

Plate counts made from broth cultures undergoing lysis, showed a progressive decrease in bacterial numbers until the period of secondary growth. At no time was there a complete disappearance of the bacteria. Attempts to produce lysis on solid media by streaking the sensitive organisms and the agent on the surface of plates have failed. Sub-

surface colonies developing in the presence of the agent, however, exhibited a moth-eaten appearance and characteristic lytic areas.

Both the sensitive strain and the resistant strain exhibited all the characteristics of the red clover bacteria and produced nodules on red clover. Both the sensitive and resistant strains have been carried through one plant passage and the organisms reisolated have proved true to original type.

5. Non-Reciprocal Interchangeability of the Wood's Clover and Garden Bean Nodule Bacteria. O. H. Sears and F. M. Clark, Agronomy Department, Illinois University, Urbana, Ill.

Detailed studies have confirmed previous observations that the nodule bacteria from legumes in each of 11 cross-inoculation groups do not produce nodules upon Wood's Clover (*Dalea alopecuroides*).

Pure cultures of Wood's Clover nodule bacteria produce nodules in abundance upon the garden bean (*Phaseolus vulgaris*). Pure cultures of organisms isolated from the garden bean nodules infect the dalea plant, only in case the bean inoculation was produced by dalea nodule bacteria.

When judged by nitrogen fixation, the garden bean culture was more effective upon the garden bean plant than the dalea culture, even though each culture produced abundant nodule development.

6. The Effect of Plant Passage on Certain Strains of the Root Nodule Bacteria. O. N. Allen and I. L. Baldwin, University of Wisconsin, Madison, Wis.

In the course of the present study, root nodule bacteria of alfalfa *Rhizobium meliloti*, 2 strains, red clover *Rhizobium trifolii*, 3 strains, pea *Rhizobium leguminosarum*, 3 strains, and soybean *Rhizobium japonicum*, 2 strains, were carried through repeated passages in the host plant to study the changes induced in the microörganisms with respect to their ability to benefit the host plants. Pure cultures of Rhizobia were used which had been carried in the laboratory on a yeast water mannitol medium for a number of years without detectable changes in their characteristics. After each plant passage new inoculations were made from a suspension of a crushed nodule. With each species 4 plant passages were secured with two or more strains.

Work was conducted in the greenhouse under controlled conditions and every effort was made to prevent contamination. The seeds were

sterilized in hot water and 1:500 solution of mercuric chloride and inoculated with the organisms immediately before being planted. Triplicate pots of each treatment were carried with frequent controls. In many cases the inocula were secured from nodules of plants grown in cotton-stoppered glass bottles. In measuring the effectiveness of the organism to improve plant growth, the plants were always grown in open pots of sterilized sand. Sterilized distilled water was used to water the plants and the mineral salts necessary for plant growth were made available in sterilized Crone's solution.

In considering the effectiveness or efficiency of the nodule organisms with respect to their ability to aid the growth of the host plants, two factors must be taken into consideration.

(1) The parasitizing effect of the organism on the host, and

(2) The ability of the organism to supply fixed nitrogen compounds to the host plants. Thus the efficiency of any particular organism is a resultant of these two opposing factors and the effects of plant passage on any strain must be studied from both angles.

With certain strains of the organisms the efficiency was increased by plant passage, while with other strains it was decreased. In general each plant passage, within the limits of this investigation, increased the effectiveness of the poorer strains of the organism. The luxuriance of growth of the host plant was increased; there was a gradual increase in the dry weights of the plants and fewer but larger nodules were formed, which were located on or near the upper portion of the tap root. Pure cultures of the better strains of root nodule bacteria showed the reverse. Apparently this decrease in efficiency was due to an increased parasitizing effect rather than a loss of nitrogen fixing ability.

Additional study to see whether these induced characteristics are temporary or permanent under the usual conditions of laboratory maintenance are now in progress.

7. *The Use of Asparagus Extract in Media for the Cultivation of Legume Nodule Bacteria.* W. R. CARROLL, University of Florida, Gainesville, Florida.

A water extract of asparagus has been used as a medium for the cultivation of legume nodule bacteria with good results. The extract compares favorably with yeast water as an accelerator. Mannitol agar containing a small amount of asparagus extract serves well as test medium for *B. radiobacter* contamination.

8. The Gum Produced by the Root Nodule Bacteria of the Leguminosae. E. W. HOPKINS, W. H. PETERSON AND E. B. FRED, University of Wisconsin, Madison, Wis.

The work reported last year has been extended to include the gum of the root nodule bacteria. Three strains of heavy gum producing root nodule bacteria were used for the study. *Rhizobium trifolii*, *Rhizobium leguminosarum*, and *Rhizobium meliloti* were grown in pure culture in synthetic media, the bacteria removed by centrifuging and the gum precipitated with acetone. It was dried to constant weight and ground to an impalpable powder. The analyses made included moisture, ash, uronic acid and pentosans. The uronic acid was determined by the method of Ling and Nanji, and the pentosans by the method of Youngburg. The uronic acid varied between 4.1 and 25.3 per cent, the lower values being obtained when no calcium salts were present in the media. The pentosan content varied between 4.3 and 16.4 per cent, the lower values again being obtained when no calcium salts were present in the media.

Glucose had previously been identified as a product of hydrolysis of the gum of *Rhizobium meliloti*. Osazones were prepared from the sugars resulting from the hydrolysis of the gums of *Rhizobium leguminosarum*, and *Rhizobium trifolii*. These osazones had melting points identical with the value of glucosazone, (204°C.). The fermentation of the hydrolyzed sugar by galactose and non-galactose fermenting yeasts gave identical fermentations. This was additional evidence that the sugar was glucose. A fermentation of the hydrolyzed gum by arabinose and xylose fermenting strains of lactic acid bacteria failed to show the presence of a pentose.

9. The Cultural Differentiation of B. radiobacter (Beij.) and Closely Related Organisms. H. E. SAGEN, WILLIAM H. WRIGHT AND A. J. RICKER, University of Wisconsin, Madison, Wisconsin.

The marked similarity of the cultural and morphological characteristics of *B. radiobacter* (Beij.), *Bact. tumefaciens* (Smith and Town.) and many of the legume root nodule bacteria, as well as the occurrence of all of them in the soil, has been the cause of considerable confusion when their separation, by cultural means, has been attempted.

Cultural studies of three groups of these organisms, using single cell cultures of *Bact. tumefaciens* as a basis of comparison, have revealed differences in respect to: (1) motility; (2) morphology; (3) absorp-

tion of dyes; (4) reduction of nitrates; (5) serological properties; (6) growth in litmus milk; and (7) growth on glycerophosphate media.

Of these differences, the growth on glycerophosphate media is the most characteristic. In this medium with manitol as the source of carbon, radiobacter strains produced abundant growth along with a conspicuous brown halo development around streaks on agar plates. This was accompanied by a whitish precipitate in the agar near the streak. *Bact. tumefaciens* when grown on the same medium produced no brown halo or precipitate.

The radiobacter cultures used did not absorb Congo red while all of the typical crowngall cultures did. The closely related organisms of crowngall which more frequently cause hairyroot in plants, did not absorb Congo red to any marked degree and refused to grow on glycerophosphate media.

In litmus milk cultures of several weeks incubation at 28°C. the hairyroot organisms produced enough acid to turn the litmus and it was often reduced with coagulation of the casein of the milk. None of the radiobacter or crowngall cultures changed the reaction of the milk and it remained a characteristic grayish brown color with a conspicuous serum zone.

Many attempts were made to get the cultures of the typical crowngall organism of single cell origin to show true motility in several liquid culture media but the movement was always observed to be Brownian. The radiobacter strains were the most actively motile and often showed longer cells than the crowngall or hairyroot organisms. The latter strains were demonstrated to have one polar flagellum often attached at a corner of the cell. Flagella have not been demonstrated on the typical crowngall cultures of single cell origin.

Serological studies of many strains of the organisms in all three groups showed no inter-agglutination relation of the groups when tested by both the tube agglutination and short methods. The true crowngall strains were homologous in the same way as the hairyroot and radiobacter strains, there being no splitting up of any of the strains within a group into subgroups or any tendency to cross agglutinate.

10. Use of the Microscope in Studying the Activities of Bacteria in Soil.

H. J. Conn, New York Agricultural Experiment Station, Geneva, N. Y.

The microscopic method for studying bacteria in soil was first proposed in 1917. It was intended at the time as a method of checking up

other procedures for counting bacteria in soil and as a means of determining the morphological types of microörganisms present. Recently Winogradski has employed the method (with slight changes in technic) for observing the kinds of organisms stimulated by addition of various ingredients to soil. The writer in following the line of investigation suggested by Winogradski's work has found another use of the method which promises to be fully as important as those for which it has previously been employed—namely for studying the food requirements of bacteria in soil.

It has been recognized for some time that the behavior of organisms in soil and in laboratory media may be very different. Without question it is more important to know their behavior in soil, but the difficulty of studying their physiology in soil has kept investigators from making much progress in this line. The microscopic method, however, offers a means of studying them. A soil may be selected in which bacteria (or at least some particular organism under investigation) do not grow. Various nutrient materials may be added to different lots of soil and each lot sterilized in test tubes. Then the various tubes may be inoculated with an organism to be studied, and its ability to grow may be learned by microscopic examination. By using this method, interesting information has already been obtained about one previously unknown but very abundant organism in soil, and further data are beng collected about others.

11. Preliminary Bacteriological Studies on Two Acid Soils. NATHAN R. SMITH AND HARRY HUMFELD, U. S. Department of Agriculture, Bureau of Chemistry and Soils, Washington, D. C.

The soils used in these studies were a Leonardtown clay loam, pH 4.6 and a Collington fine sandy loam pH 5.2. Enough of the uncultivated soil to fill eight sections was brought into the greenhouse and placed in benches. The treatments for each soil were as follows: first series—control; limestone only; green rye only; green rye and limestone; second series—control; limestone only; green vetch only; green vetch and limestone. Soil samples were taken before treatment, 2, 4, 7, 14, 21, 35, and 49 days after treatment, and brought into the laboratory for analysis.

The numbers of fungi and actinomyces in both soils remained very constant throughout the experiment regardless of treatment. The pH of the soil with rye green manure fluctuated only slightly, whereas in the case of vetch green manuring there was a tendency for the soil to become

slightly more acid. The amount of nitric nitrogen and the total counts were greatly affected by the treatments. There was a sharp rise in the total counts on the second and fourth days followed by a sharp decline where a green manure with lime was added. This was followed by a gradual rise. In the case of green manure without lime, there was an initial increase in the Collington soil followed by a decrease. However, in the more acid Leonardtown soil there was no initial increase and the numbers remained quite constant although the nitrates increased from 10 to 80 ppm. As a rule, in the early stages of decomposition, nitrates tended to rise with the total counts and after the 7 day sampling, to vary inversely with the total counts.

Small Gram-negative rods were responsible for the greater part of the increases in total counts noted above. The Gram-positive bacteria increased considerably during the first few days after the turning under of the organic matter; they belonged largely to the coccoid and pseudodiphtheroid groups.

The outstanding fact of these experiments is the lack of increase in the numbers of fungi in these acid soils when green rye or vetch is turned under 4 to 5 inches in a greenhouse bench, and the rapid increase in numbers of bacteria on the second and fourth day after treatment.

12. A Practical Method for Determining the Number of Clostridium butyricum Colonies in Acid Soil. HAROLD W. BATCHELOR AND IRVIN H. CURIE, Ohio Agricultural Experiment Station, Wooster, Ohio.

The method is essentially a modification of the Winogradski silicic acid plate method for determining the number of *Azotobacter* colonies in soil. The same inorganic nutrients as recommended by him are used but the calcium carbonate is increased to 2 grams per liter. One per cent glucose is substituted for mannitol and 1.5 per cent Bacto-Difco agar is substituted for the silicic acid.

Seventy-five cubic centimeters of the nutrient agar are pipetted into aluminum Petri dishes having a diameter of 20 cm. and allowed to stand for four to five hours. One-half gram of a soil separate which passes a 20 mesh sieve but does not pass a 40 mesh sieve is distributed uniformly over the solidified agar and allowed to stand in contact with it until the particles are moistened. 100 cc. of the nutrient agar at a temperature of 75 to 80°C. are then pipetted over the particles and allowed to solidify. The plates are incubated at 28°C. for six days. The gas produced through the fermentation of the glucose by the anaerobic bacteria results in the formation of gas bubbles and fractures in the agar around

the soil particles. These fractures are counted with the aid of a brilliant light directed on the surface of the plate. The colonies may be stained by pouring 15 cc. of Lugol's solution over the plate and allowing it to diffuse through the agar. The older colonies containing the typical clostridium forms are stained a deep blue, and the younger colonies a reddish-brown.

The method may be used to determine the presence of both *Azotobacter* and *Clostridium* colonies on the same soil particle by using a double incubation-double pour method. The soil is distributed over the first layer and incubated four days. The second layer of agar is then poured and allowed to solidify. The *Azotobacter* colonies are then counted and identified with a dot of black india ink on the surface of the agar. The plates are then incubated a second time for 6 days and the fractures counted with the aid of red ink.

Data obtained by the method are presented. Limitations of the method and precautions to be observed are discussed.

13. Some Influences of the Development of Higher Plants upon the Abundance and Activities of Microörganisms in the Soil. R. L. STARKEY, Department of Soil Chemistry and Microbiology, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Some of the influences of the development of higher plants upon the microörganisms of the soil have been observed by periodically determining the abundance of certain groups of the organisms and measuring the rates of certain soil processes. In general, the results indicate that plant development brings about a greater development of microörganisms of all kinds about plant roots. The increase is slight in the early stages of root growth, becomes greatest as plants approach maturity and become less pronounced subsequent to death of the plants. The influences of plant development are greatest upon the *Radiobacter* group of bacteria but also are striking upon the bacterial population as a whole. The influences upon actinomyces are not very pronounced. Measurements of the abundance of filamentous fungi as determined by plate counts show no significant consistent influence of plant growth upon their prevalence. The abundance of nitrogen fixing bacteria was not noticeably affected by plant development as measured by growth from dilutions of the soils inoculated into glucose solution. The carbon dioxide evolved from soils obtained from the rhizosphere was greater than that from fallow soils. The greatest production of carbon dioxide accompanied advanced plant development and early degenera-

tion. Nitrification of the soil's own nitrogen was somewhat greater in the planted soils with the advancement of plant growth. In a similar way, the greater the extent of plant development the greater was the influence of the planted soils on accelerating nitrification of added ammonia-nitrogen. In the planted soils there was a greater tendency for consumption of ammonia and nitrate nitrogen suggesting the presence of appreciable amounts of available organic matter of low nitrogen content.

Different plants affected the abundance of organisms and their activities differently but, wherever plant growth was appreciable, marked effects were apparent. The legumes studied did not show any more pronounced effects than some of the non-legumes. The influences of rape, corn and mangel beets were greater than those of beans, sweet clover, table beets, oats or potatoes.

14. Biochemical Aspects of the Acetone Butyl Alcohol Fermentation of Corn Meal. W. H. PETERSON, E. B. FRED AND H. R. STILES, University of Wisconsin, Madison, Wis.

Development of bacteria, degradation of carbohydrates and proteins, and formation of products were determined simultaneously on a single large culture (40 liter culture in a 50 liter Pyrex flask). The maximum number of bacteria (1 to 2 billion per cubic centimeter) is reached in about 30 hours. The increase in numbers of bacteria is accompanied by a rise in the titratable acidity and closely followed by the production of solvents (acetone, butyl alcohol, and ethyl alcohol). A decrease in the number of bacteria and fall in acidity occur simultaneously with the maximum rate of solvent production. All of the solvents are produced simultaneously, but some evidence was obtained that periods of high acidity are characterized by increased acetone and decreased ethyl alcohol production.

The starch content of the medium decreases progressively throughout the fermentation. Reducing sugar reaches a maximum at the end of forty hours and then decreases rapidly. The sugar has been identified as glucose.

Hydrolysis of protein is a concurrent change with acid and solvent production. About two-thirds of the total nitrogen is made soluble. This consists of protein, proteoses, peptones, and amino acids.

The total gas produced during the fermentation consists of about 60 per cent (by volume) of CO_2 and 40 per cent of H_2. During the first three hours it is largely H_2 but after fifteen hours the percentage of CO_2 is greater than that of H_2.

15. The Acceleration of the Alcoholic Fermentation of Cane Molasses by the Use of Vegetable Carbons and Other Inert Substances. WM. L. OWEN AND W. P. DENSON, Department of Bacteriology, Louisiana Experiment Station, Baton Rouge, La.

The alcoholic fermentation of molasses solutions can be greatly accelerated by the addition of small amounts of vegetable carbons. The accelerating effects of these substances while quite pronounced in solutions of ordinary density, is especially so in those of higher density. This acceleration appears to be partly due to the more rapid liberation of CO_2 in the presence of the carbons, but the increased rate of the reduction of acetaldehyde on the carbon surfaces seems to play an important part, as does also the stimulating effect of the carbons upon the yeast themselves. The latter effect is partly to be attributed to the adsorption of toxic substances from the medium. The minute amount of these accelerating substances that is required to produce the stimulating effect upon fermentation suggests a catalytic action, and the fact that substances carrying positive charges are more active in this capacity than negatively charged colloids indicate that the nature of the charge determines to a large extent the value of these substances as accelerators of fermentation. Since the fermentation of some of the hexose sugars is accelerated to a greater extent than others by the presence of carbons an interaction between these substances and the different sugars is indicated. A prior filtration of the molasses solutions through carbons tends to prevent any acceleration of the fermentation of the former when treated subsequently with the latter. The accelerative action of these substances seems greater under conditions where the H-ion concentration is below that required for the optimum rate of fermentation.

16. Changes of the Endpoint of Fermentation by Very Large Seeding. OTTO RAHN, Bacteriology Laboratory, Cornell University, Ithaca, N. Y.

The endpoint of a fermentation, i.e. the maximum amount of products formed in a certain medium, is ordinarily quite constant. It can be increased to a certain extent by increasing the number of acting organisms above the number normally developing in the medium.

By adding large amounts of yeast to a sugar solution very rich in protein, the final amount of alcohol could be increased from the normal 12.0 to 13.9 per cent; in the same way, the amount of CO_2 formed increased from 11.0 to 12.6 per cent of the weight of the medium. This

was accomplished by adding as much as 10 grams of yeast to 100 cc. of medium.

Some streptococci in milk are known to be favored by addition of peptone while others are not. In the first case, the streptococci grow to larger numbers, and produce considerable more titrated acid. A strain of *Str. bovis*, produces 0.73 per cent of lactic acid in milk, and 1.00 per cent in milk plus 1 per cent peptone. This increase is too large to be accounted for by the buffer action of the added peptone. It was not always possible, to establish in this case the same principle as with yeast. The cells from 3000 cc. of a lactose broth culture of the same streptococcus, removed by supercentrifuge and added to 200 cc. of fresh lactose broth, produced the same amount of acid as a normal culture. In milk cultures, large cell quantities gave higher titrated acidity as well as higher [H] ion concentration.

17. Nuts as a Possible Source of Escherichia coli Found in Candy. JOHN WEINZIRL, University of Washington, Seattle, Wash. (Presented by RACHEL E. HOFFSTADT.)

Examinations of 600 samples of nuts of 7 different kinds were made for the presence of *E. coli*, the purpose being to determine whether nuts might be a source of contamination with this organism when incorporated into candy. The samples were secured from a wholesale manufacturer of candy, and retail shops. *E. coli* was found to be present on 0.5 to 2 per cent of the samples, a percentage slightly lower than that found on candy. These results do not indicate that nuts are an important source of the *E. coli* found on candy; rather, they seem to show that, like candy, they are contaminated by the hands of the vendors.

18. Discoloration of Halibut. F. C. HARRISON AND W. SADLER, McGill University, Montreal, Canada.

Over 50 million pounds of halibut are landed yearly on the Pacific Coast. Many of these fish are discolored on the white or belly side. The color is a greenish-yellow. Microscopic examinations of the discolored slime showed numerous bacilli, mostly gram negative, also cocci gram positive and negative. Isolation revealed the presence in large numbers of *Pseudomonas fluorescens*. Pure cultures of the organism caused the typical discoloration on clean fish. The organism was found in large numbers in the artificial ice used for packing the fish in

the boats. Ice all obtained from upland surface waters. This organism also present in large numbers in bilge water (150 million per cubic centimeter) on bins, hold, landing net, etc.

Halibut fresh from the sea, and before being pulled into boat, had no *Ps. fluorescens* on skin or in slime but did have numerous other organisms present. Sea water obtained at depth of 30 to 50 fathoms contained no *Ps. fluorescens* but bacteria present in small numbers. Over 100 isolations of *Ps. fluorescens* were made from as many different boats and various strains studied. Organism grew well at temperatures of 1.5 to 5°C.

19. Organisms Causing Spoilage of Tomato Products. CARL S. PEDERSON, New York Agricultural Experiment Station, Geneva, N. Y.

An examination of 83 samples of various spoiled tomato products from various sources and the isolation of the causative organisms has shown that the spoilage of such products is usually caused by certain species of lactic acid producing bacteria. Only 5 yeast cultures and 4 cultures of sporeformers have been isolated. All other cultures have been identified as belonging to one of the following species, *Lactobacillus lycopersici* Mickle, *Bacterium gayoni* Müller-Thurgau and Osterwalder, *Lactobacillus pentoaceticus* Fred and Peterson, *Bacterium mannitopoeum* Müller-Thurgau and Osterwalder, the so-called *Bacillus pleofructi* Savage and Hunwicke or *Lactobacillus plantarum* (Orla-Jensen) Bergey.

Each of the above species has distinct morphological and cultural characteristics by which they can be distinguished from one another. Morphologically all are rods except *Bacillus pleofructi* which ordinarily is a coccus but lengthens at times into rods. It is not a spore-former so that the genus name assigned to it is unsuitable. It is related to the genera *Leuconostoc* van Tiegham and *Betacoccus* Orla-Jensen. The thermal death point of the entire group is relatively low and they are usually killed in the ordinary canning process.

The 5 gas producing species form mannitol from fructose as well as smaller amounts of alcohol, lactic acid, and carbon doxide. Lactic acid and acetic acid are the major end products from the fermentation of pentoses. Lactic and acetic acids, carbon dioxide and alcohol are produced from glucose.

A detailed report will appear as a bulletin from the New York Agricultural Experiment Station.

20. The Scope and Findings of Field Work During the Canning Season of 1928. E. J. CAMERON, Research Laboratories, National Canners Association, Washington, D. C.

In 1926 and 1927 studies were made to determine causes leading to specific outbreaks of thermophilic spoilage in canned peas and corn. This work led to the establishment of approximate quantitative standards for the three groups of thermophilic spore-bearing organisms most often concerned in spoilage of non-acid products. Included in this category are (1) the flat sour bacteria causing spoilage through the production of acid without gas, (2) thermophilic anaerobes causing spoilage through the production of both acid and gas, other than H_2S, (3) the so-called sulfide spoilage bacteria (*Cl. nigrificans*, Werkman and Weaver), also thermophilic anaerobes, except in this instance the gas produced is hydrogen sulfide.

From the results of tests on samples representing various stages in the canning process, it was possible to state whether a so-called spoilage hazard existed at the time of sampling, and by a series of such tests to state whether the situation was such as to point to the possibility of future difficulty.

With a view to applying such knowledge as existed on as wide a scale as would be practicable, a large motor truck was equipped as a field laboratory. Culturing operations, incubation and culture readings were done on the truck. The preparation of media, wet and dry sterilizaton and dish washing was done by means of portable equipment which could be carried with the truck and installed in an appropriate part of the cannery where the laboratory for the time would be located. To facilitate matters, basic media were prepared and canned in the Washington Laboratory in advance of the season.

By means of this mobile outfit a field crew of five men were able to collect and examine samples from approximately 125 canning factories in Wisconsin, Minnesota, Iowa and Nebraska. This work was done by establishing the truck laboratory in canneries which were central in the various canning sections and samples were collected from outlying canneries by automobile.

Earlier findings were confirmed. Sugar appears as the outstanding source of thermophilic spoilage bacteria to account for the initial infection of canning equipment and may also at times constitute a direct spoilage hazard. The raw product is an entirely secondary source of spoilage organisms. Wooden tanks used for heating non-acid liquids

may become seeded and infect the canned product. In general, any part of the canning equipment subjected to heat may be involved.

During the course of this work, spoilage hazards were found to be extremely rare. It was found, however, that certain elements of equipment rather often permitted the establishment of thermophilic contamination. Among these should be mentioned the blanching equipment in the canning of peas and pre-cooking apparatus used in canning corn.

21. Growth of Cl. Botulinum in "Waterless-Processed" String Beans. LAWRENCE H. JAMES, Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

Vegetables canned by the "waterless process" method are not immersed in a liquid, as water or brine, but are filled into the containers, the air removed by a vacuum pump or replaced by steam, the containers sealed and processed. Cans of "waterless processed" vegetables contain no liquid phase in which bacterial contamination might readily spread throughout the container. One bean, infected with *Cl. botulinum* spores, was placed in the center of the top layer of a series of cans of string beans packed by "waterless processing." After incubation the toxicity of the beans at the top, middle, and bottom levels was tested by guinea-pig feeding. The spread of *Cl. botulinum* through string beans so canned was found to be as complete as in vegetables canned in brine. The spread of the organism under such conditions was also demonstrated progressively along a row of adjacent beans placed in a petri dish and incubated under anaerobic conditions. Toxic cultures were isolated from all the beans of the row. The pieces of string beans were also toxic. The entire contents of a can of "waterless processed" string beans may become toxic from the presence of a single infected bean.

22. Studies on Lactic Acid Streptococci. II. On Streptococcus lactis L. and its relation to the fecal streptococci. KARL J. DEMETER, Forschungsanstalt für Milchwirtschaft in Weihenstephan, Germany.

One hundred and six streptococci from milk and fecal sources (man, calf, and insects) were examined. The results of Ayers and his co-workers on *Str. lactis* could be confirmed almost in every sense. This holds also for the fecal streptococci, which gave the same percentage

concerning fermentation of certain carbohydrates. The "order of availability" is the same for the lactic and fecal streptococci. Of the fecal streptococci, about 75 per cent conform to Dible's central type of the enterococcus and its three variants. This is true also with the lactic streptococci. The percentage of Ayers' Var. A (production of gas and ammonia from peptone) and B is the same for the fecal strains as for the lactic strains.

The fermentation of mannite(Andrewes and Horder), of arabinose (Orla-Jensen), blackening of aesculin broth (Meyer and Schonfeld), and the ability to sour milk at 50°C. (Orla-Jensen) do not differentiate the fecal from the lactic streptococci. The litmus milk reaction is of no value either, because the majority of those fecal streptoocci, which at first did not give a typical reaction, did so after being kept in artificial medium (milk) for months or years. This shows clearly that the *Str. lactis* originally may be derived from the intestines and that it still is originating from there. There is no regularity regarding the formation of volatile acids within the lactic strains nor within the fecal strains.

We do not hesitate to say that from the data obtained there is no real difference between the *Str. lactis* and *Str. faecalis* (Enterococcus) and that the name "*Str. lactis*" may be used only to define a local form of the *Str. faecalis*, more or less adapted to the milk medium.

23. Relationships of the Various Acid-Proteolytic Cocci. G. J. Hucker, New York Agricultural Experiment Station, Geneva, N. Y.

Twenty-five strains of acid-proteolytic cocci were secured from various laboratories and studied to determine the relationships of the types which have been described, some of which have been stated to play an important part in both the normal and abnormal changes in ripening cheese and other dairy products.

It was found that there are two distinct types of acid-proteolytic cocci being studied in various laboratories. One type comprised strains which proved to be micrococci. Belonging to this group were *Micrococcus caseolyticus* Evans 1916 (*M. casei* Hucker 1924), *Tetracoccus casei* Orla-Jensen 1919, *T. liquefaciens* Orla-Jensen 1919, as well as *Mammococcus*, *Enterococcus*, *Caseococcus*, and *Gastrococcus* described by Gorini 1926.

The remaining types comprised strains of streptococci which included *S. liquefaciens* Orla-Jensen 1919 and many of the acid-proteolytic types studied by Gorini. It is possible that all the gelatin liquefying strep-

tococci belong to one species with *S. liquefaciens* Orla-Jensen 1919 as the central type.

The acid-proteolytic cocci should not be considered from a systematic standpoint as a separate genus. Many of the organisms of this type being studied in bacteriological laboratories are true streptococci, while certain others are cocci identical with well-recognized and common species of the genus *Micrococcus*.

More detailed information may be found in New York State Agricultural Experiment Station Technical Bulletin No. 144.

24. Reduction of Janus Green B in Milk. H. R. THORNTON, Department of Bacteriology, North Dakota Experiment Station, Fargo, North Dakota.

Workers in Europe have recommended the substitution of janus green B for methylene blue in the reduction test in milk. Janus green B reduces in milk to a red or pink compound thought to be a safranin. This reaction is considered irreversible. This compound further reduces to a leuco-base, which reaction is reversible.

Electrometric studies show that both these reactions take place over potential ranges more negative than that at which methylene blue reduces.

In 157 samples of milk from widely scattered sources the janus green reduction times, with one exception, were greater than the methylene blue reduction times. In one sample the reduction times were the same. The differences varied from an average of thirty-five minutes in milks reducing methylene blue in less than one hour to an average of eighty-three minutes in milks reducing methylene blue in six hours or over. This is accounted for by the difference in the potential ranges over which the two dyes reduce, and by the marked poising action of janus green B.

The endpoint of reduction of janus green B in milk is more indefinite than that of methylene blue. This is due to the poising action of the former and to the difficulty ensuing from the mixture of colors, i.e., the blue of the janus green, the red of the reduced compound, and the white of the milk.

The rate of diffusion of atmospheric oxygen into tubes of whole milk is not rapid enough to have a measurable effect upon the reduction times of methylene blue, janus green B, or litmus. Janus green B showed no advantage over methylene blue as an indicator of quality in milk but several disadvantages were observed.

25. The Bacterial Flora of Pasteurized Milk. H. A. HARDING AND A. R. WARD, Mathews Industries, Inc., Detroit, Mich., AND H. G. HARDING, Akron Pure Milk Co., Akron, Ohio.

It is generally agreed that tubercle bacilli are the most heat resistant of any of the pathogenic bacteria which find their way into the milk supply. The studies of Park have shown that milk heavily seeded with tubercle bacilli is rendered innocuous by heating to 142°F. for ten minutes. Commercial milk pasteurization, as it is almost universally carried on, consists of heating the milk to a minimum of 142°F. for a minimum of thirty minutes. This is three times the treatment time required to totally destroy any pathogenic bacteria which may be present. Accordingly, except when infected after the treatment, time pasteurized milk has no pathogenic flora. Early studies of pasteurized milk were conducted mainly upon an agar medium prepared from Witte peptone, Liebig beef extract and shreaded agar, with or without lactose, and with a reaction of 1.5 to the Fuller scale. The bacteria growing upon such agar suffer a marked reduction in numbers during pasteurization. This reduction is not strictly proportional among all types. It is greater among the non-acid forming bacteria with the result that the proportion of these acid forming bacteria is greater in pasteurized milk than in the raw milk before pasteurization. Incident to war conditions, the use of a variety of peptones and of beef extracts in preparing agar media with a wide range of reaction led to the discovery of a thermophilic flora in pasteurized milk. Study of this thermophilic flora has been handicapped by the lack of convenient methods for recognizing the presence and the relative numbers of its representatives. In 1923 Harding and Ward reported that the presence of some representatives of this thermophilic flora could be shown by incubating standard agar plates at 62.5°C. (145°F.). The methylene blue reductase test conducted at 100°F. is being widely used to determine the presence and relative numbers in raw milk of the flora which thrives at 100°F. We have found that the presence and relative numbers of the thermophilic flora in pasteurized milk can likewise be determined by the application of the methylene blue reductase test by carrying out this test at 145°F. (62.5°C.). This same methylene blue test at 145°F. can be used to determine the presence of thermophiles in raw milk. They have been found in all samples of raw milk yet examined, though the numbers present vary widely. Entering the pasteurizing machinery with the raw milk, the thermophilic bacteria attach themselves to the walls of the apparatus in which the milk is held at pasteurizing tempera-

ture and there multiply rapidly. As the pasteurizing process is continued these thermophilic bacteria pass out in increasing numbers with the pasteurized milk. Direct microscopic examination of the pasteurized product shows a steady and rapid increase in this thermophilic flora from approximately 1,000,000 per cubic centimeter early in the process to many millions when the pasteurizing process has been continued for six or more hours. There is no evidence that the presence of this flora has any sanitary significance and except under unusual conditions it does not have any commercial significance.

26. Thermophilic Spore-Formers Associated with Milk. PAUL S. PRICKETT, New York Agricultural Experiment Station, Geneva, N. Y.

In studying the problem of unusually high counts from pasteurized milk a total of 480 cultures of thermoduric (heat-resistant) and thermophilic (heat-loving) organisms have been secured from a variety of sources. This total includes seven types of organisms which are listed in the order of their importance based on the number of times each type was isolated in this study: Spore-forming rods, streptococci, non-spore-forming rods, micrococci, *Actinomyces*, sarcinae, and a yeast.

The cultures of facultative and obligate thermophilic spore-formers isolated in this study were divided into 10 groups or species based on their morphological, cultural and physiological characteristics. The names assigned to the 10 groups or species are as follows: *Bacillus subtilis* Cohn, as described by Gottheil and Ford (not a true thermophile) *B. terminalis* Migula var. *thermophilus* Prickett; *B. michaelisii* Prickett (probable synonym, *B. thermophilus-aquatilis-liquefaciens* Michaelis); *B. calidus* Blau; *B. thermoalimentophilus* Weinzirl; *B. aerothermophilus* Weinzirl; *B. thermoliquefaciens* Bergey; *B. nondiastaticus* Bergey; *B. calidolactis* Hussong and Hammer; and *B. kaustophilus* Prickett.

The results of this study and the results of previous studies by other workers indicate that the majority of the thermophilic spore-formers are characterized by being aerobic, and by having terminally located ellipsoidal to cylindrical spores as well as by their thermal relations. Of these 10 species, only 2 were found to have centrally located spores, namely: *Bacillus nondiastaticus* Bergey, and *B. subtilis* Cohn. Under special conditions these spore-forming bacteria may occur in pasteurized milk in enormous numbers.

A complete report of this work has just been published as Tech. Bull. No. 147 of the New York Agricultural Experiment Station, Geneva, N. Y.

27. The Significance of Thermophilic Spore-forming Bacteria in Pasteurized Milk. ROBERT S. BREED, PAUL S. PRICKETT AND M. W. YALE, New York Agricultural Experiment Station, Geneva, N. Y.

Investigations by the authors have shown that truly thermophilic bacteria rather frequently find conditions favorable for growth during the pasteurization of milk, particularly in large plants where the pasteurizing equipment is kept continuously in operation for from three to twelve hours or even longer. The significance of the presence of these organisms is largely dependent upon their origin, upon the conditions under which they grow in the milk and upon the effect of their activity in milk.

Organisms of the types present in pasteurized milk are almost universally distributed in soil. They are also universally present in hay, grain, silage and manure, being associated with the heating of these materials. As they usually form spores, it is natural to find these spores present in the dust and similar materials of the cow stable. Even the cleanest milk may occasionally contain small numbers of thermophilic bacteria.

The only dairy utensil that offers favorable conditions for these bacteria seems to be the milk can as these frequently stand exposed to the sun for a sufficient length of time to be heated to temperatures favorable for the growth of thermophilic bacteria. Tests have shown that these organisms may occur in milk cans.

It does not seem probable that especially favorable conditions for growth occur normally before the milk reaches the pasteurizing plant. During pasteurization, however, there are many opportunities offered for their growth as for example in (*a*) foam which may remain in the pasteurizing vat for hours; (*b*) in milk cooked onto the metal pipes or other equipment where the water or steam used in heating the milk is at a temperature above 150–152°F.; (*c*) dead ends which cause eddies in the current of hot milk; (*d*) gaskets, pitted metal surfaces and other rough places which offer conditions favorable for the development of these bacteria.

During pasteurization, delays caused by lack of milk at the receiving platform, lack of bottles at the bottler or other things cause milk to be held longer than thirty minutes at pasteurizing temperature and so give a chance for the growth of the thermophilic bacteria. Another rather common practice in milk plants is to repasteurize milk returned from delivery wagons or milk drained from the heater, holder or bottler at the end of the day's run. All of this milk is apt to contain large numbers of

thermophiles and serves to inoculate the fresh milk with thermophilic bacteria.

The chief effect of the presence of millions of the thermophilic bacteria per cubic centimeter is to cause a change in the reaction of the milk which is usually indicated by an increased acidity, although some of these thermophilic types are liquefiers and alkali producers. A number of large milk plants report that the growth of these bacteria affects the flavor of the milk. Both of these characteristics make their presence highly undesirable from the commercial standpoint. As their presence in large numbers is due largely to favorable temperatures, these bacteria do not have an intimate connection with unsanitary practices. It is self-evident also that obligate thermophilic bacteria cannot be pathogenic in the ordinary sense as they do not grow at temperatures as cold as those found in the human body. Likewise there is no evidence that indicates that facultative thermophilic bacteria are pathogenic.

Detailed reports of this investigation have appeared or will appear as bulletins of the New York Agricultural Experiment Station.

28. The Effect of Heat Produced by an Alternating Electric Current on Tubercle Bacilli in Milk. C. M. CARPENTER, Diagnostic Laboratory, New York State Veterinary College, Cornell University, Ithaca, N. Y.

Raw and pasteurized milk heavily inoculated with human and bovine tubercle bacilli was subjected to a 220-volt 60-cycle alternating current by pumping the milk between two carbon electrodes. The milk passing between the electrodes completed the circuit and, by its resistance to the current, raised the temperatures of the milk to 150°F., 155°F., and 160°F. Guinea pigs injected with milk treated in this manner showed no evidence of tuberculosis when the milk was heated to 155°F. and 160°F. Some of the guinea pigs injected with milk treated at 150°F. developed typical lesions of the disease.

29. The Bacterial Count of Ice Cream Held at Freezing Temperatures. JOHN WEINZIRL AND ALICE E. GERDEMAN, Department of Bacteriology, University of Washington, Seattle, Washington. (Presented by Rachel E. Hoffstadt.)

Thirty-four samples or bricks of ice cream from two producers were kept at temperatures below zero centigrade and examined for numbers of bacteria per gram on alternate days. Twelve samples were held at minus 3°C., 12 at minus 6°C., and 9 at minus 10°C. The results indi-

cate that storage of ice cream at minus 10°C. or above, does not prevent all bacterial multiplication or the increase of the bacterial count. The results furnish at least a partial explanation of the increased numbers of bacteria found in retailers' samples as compared with those from producers.

30. Contaminated Water as a Source of Surface Flavor in Pasteurized Creamery Butter. DONALD BETHUNE SHUTT, Laboratory of Bacteriology, Ontario Agricultural College, Guelph, Canada.

Surface flavor butter is almost unknown in butter made in city creameries, where the water supplies are known to be pure, but it would appear that this flavor is quite prevalent in creameries in the rural districts, especially after prolonged periods of wet weather.

All suspected water supplies have been found to be contaminated with *Pseudomonas fluorescens.*

Sterile butter when inoculated with this organism and incubated at 25°C. for 28 days developed typical surface flavor. Investigations in the field and laboratory proved that this flavor could be controlled by: substitution of pure water for contaminated water for butter washing, subjecting contaminated water to a temperature of 190°F. for 10 minutes, neutralizing the cream to not less than 0.35 per cent acid.

31. Outbreak of Mold in a Cheese Factory. D. H. DUNHAM, University of Wisconsin, Madison, Wis.

A factory manufacturing a soft bacterial ripened cheese known in the trade as "Liederkranz" was losing a considerable portion of its product through the profuse growth of a *Penicillium* thereon. The manufacturing operations had been recently transferred from a factory of rather primitive type to a modern structure designed for the special purpose. The equipment for maintaining the proper temperature and humidity of the curing room and the relation of the various rooms to each other were such as to force the air with its load of mold spores from the curing room into the room, known as the making room, in which the cheeses were kept for a few days following their manufacture. The condition of the cheese in the making room in regard to moisture and salt content was such as to favor the germination of the mold spores. The condition in the curing room was unfavorable for spore germination, but did not inhibit to any marked extent vegetative growth and the production of spores.

The fumigation of the various rooms was impossible without closing

the factory. The trouble was gradually overcome and finally reduced to a very low level by: (1) A general factory renovation; (2) The isolation of the making room from the curing room; (3) The sterilization of all materials such as boards and racks as they passed from the curing room to the making room to be used once more; (4) The use of a slightly lower temperature in the making room to inhibit spore germination.

IMMUNOLOGY AND COMPARATIVE PATHOLOGY

1. *Effect of Oral Administration of Ox-Bile on Appearance of Agglutinins for Esch. coli in Sera of Normal Rabbits.* La Verne Barnes, The Bacteriological Laboratories, State College of Washington, Pullman, Wash.

Besredka believes that the oral administration of ox-bile to rabbits renders the intestinal mucosa more pervious to bacterial protein. The following experiment was devised to test this theory:

Cultures of *Esch. coli* were isolated from the feces of normal rabbits. Gram negative, short rods were in predominance in samples of feces studied. The agglutination titers of the sera of 5 normal rabbits for *Esch. coli* were determined. Ox-bile, in doses of 10 cc., was given by mouth to 3 rabbits on alternate days until a total of 80 cc. had been administered. The agglutination titers were then determined at varying intervals. The last agglutination test was made approximately 90 days after the last dose of bile. No change in the agglutination titers was observed.

2. *Effect of Oral and Subcutaneous Injections of Pasteurella avicida in Rabbits Vaccinated by the Oral and Intravenous Methods.* La Verne Barnes, The Bacteriological Laboratories, State College of Washington, Pullman, Wash.

Four rabbits were given oral injections of *Past. avicida* vaccine, and 5 others were vaccinated intravenously. The agglutination titers were determined. The animals were then given doses of virulent cultures of *Past. avicida* as follows: 2 orally, and 3 intravenously vaccinated rabbits were given the test doses by mouth. The remaining animals in each group were injected subcutaneously. Controls were injected for each group. All 5 of the control rabbits died in less than 24 hours. Results in the vaccinated animals do not indicate any difference in the degree of protection by the two methods used. The results have a bearing on the theory of local immunity.

3. A Note on the Use of "Specific Wet Dressings" of Staphylococci According to the Method of Besredka. LAVERNE BARNES, The Bacteriological Laboratories, State College of Washington, Pullman, Wash.

Pure cultures of staphylococci were isolated from boils on the faces of two students. Wet dressings of the filtrates of broth cultures, as suggested by Besredka, were applied on only one side of the face to determine whether a local immunity would develop. Immunity was not developed in 24 hours as claimed by Besredka. Before any observations as to the general effect could be made, the patients had applied the filtrates to the entire face. In both cases definite improvement was reported. No attempt was made to isolate *B. acne* from these cases.

At the present time, vaccines of *Esch. coli* and *Eber. typhi* are being applied cutaneously to determine whether any agglutinin formation will take place. Negative results in this respect have been obtained to date.

4. Immunological Studies of Typhoid Vaccination by Mouth. 1. Agglutinins Formed in Persons Treated Orally with Triple Typhoid Bacterin. RACHEL E. HOFFSTADT AND RANDALL L. THOMPSON, Department of Bacteriology, University of Washington, Seattle, Wash. (Presented by RACHEL E. HOFFSTADT.)

Ninety-three persons were treated orally with liquid triple typhoid bacterin. Tests were made at weekly intervals for 11 weeks and at the end of 5 and 9 months. Only in 1 case was there a systemic response. Of those treated, 83.5 per cent showed agglutinins for typhoid and a lesser number for paratyphoid. Bile prepared individuals showed a higher percentage of agglutinins than non-bile prepared persons. The latent period for the appearance of agglutinins was 3 to 4 weeks. Titers of 600 were reached. No higher dilution was run in the tests. Maximum titers were reached in the fourth to fifth weeks. Duration of agglutinins in a fair proportion of individuals was demonstrated at the end of 5 and 9 months. Previous treatment with the subcutaneous method or a history of typhoid fever had no effect except to raise the titers.

5. Immunological Studies of Typhoid Vaccination by Mouth. 2. Complement Fixations and Precipitin Reactions in Persons Treated Orally with Bile and Triple Typhoid Bacterin. RACHEL E. HOFFSTADT AND RANDALL L. THOMPSON, Department of Bacteriology,

University of Washington, Seattle, Wash. (Presented by RACHEL E. HOFFSTADT).

Thirty-nine persons were treated orally with liquid typhoid bacterin. Eighty-eight per cent of all persons showed positive fixation and precipitins. Complement fixation tests and precipitin tests were made at weekly intervals over a period of 11 weeks and at the end of 5 months. Bile seems to have no effect on the production of complement fixations and precipitins. Latent period for the appearance of complement fixations for typhoid is 4 to 5 weeks. Duration is irregular for both fixation and precipitins. Complement fixations are demonstrated after 5 months. There seems to be a definite relationship between the appearance of fixations and agglutinins in this group but it corresponds to that reported for cases of typhoid fever.

6. *Immunological Studies of Typhoid Vaccination by Mouth. 3. Agglutinins and Complement Fixation Reactions Formed in Persons Treated with Typhoid Bacterin in Capsule.* RACHEL E. HOFFSTADT AND CARL E. MARTIN, Department of Bacteriology, University of Washington, Seattle, Wash. (Presented by RACHEL E. HOFFSTADT.)

Forty-two persons were treated orally with a dry triple typhoid bacterin in the form of a capsule. A marked systemic response was found in 2 persons only. Eighty-seven per cent of the persons treated gave positive agglutinins. Seventy-nine per cent gave positive complement fixation. The latent period for agglutinins was 3 to 4 weeks, and for complement fixations, 4 to 5 weeks. Titers of agglutinins compared well with those produced by the liquid bacterin. Persons in which the bile was given separately from the capsule showed a more ready response than those who had a capsule in which the bile was incorporated.

7. *Bacteriophage as a Substitute for the Typhoid Vaccine.* N. W. LARKUM, Michigan Department of Health, Lansing, Michigan.

Bacteriophage prepared against *B. typhosus*, *B. paratyphosus* A and B has been used for immunization against typhoid fever. About 1200 inmates of state institutions have been inoculated and the serological response in about 200 has been studied. The study has been concerned with the type of reaction following injection, the agglutination, opsonic and bactericidal response, the effect of variations in dosage, and the duration of the antibodies produced as compared with the usual vaccine. One inoculation of 2.0 cc. of bacteriphage has resulted in the production

of agglutinins to the same titre as produced by the vaccine. The bactericidal power of the blood and the opsonic index of those inoculated with bacteriophage has been higher than those produced by vaccine. After 6 months antibodies were present to a higher titre in those having a single inoculation of bacteriophage than in those having a single inoculation of vaccine, and equal to the antibody titre in those having three inoculations of vaccine. Three inoculations of bacteriophage gave less satisfactory results than did a single inoculation.

8. The Effect of Specific Sera Upon the Phenomenon of Local Skin Reactivity to B. typhosus Culture Filtrate. GREGORY SHWARTZMAN, Laboratories of Mount Sinai Hospital, New York City.

A phenomenon of local skin reactivity to *B. typhosus* culture filtrate was previously described (J. Exp. Med., xlviii, 1928–247). The reactivity was induced by skin injections of the filtrate. If 24 hours later an intravenous injection of the same filtrate was given to rabbits there appeared an extremely severe hemorrhagic necrosis at the site of previous skin injections. There were found certain features *which considered together* distinguished this phenomenon sharply from the known phenomena of bacterial allergy and the Arthus phenomenon.

Further work is reported on the relation of the specific antisera to the factors which induce the skin reactivity, so called "skin preparatory factors." The neutralization of these factors is easily accomplished by specific sera. In order to apply this phenomenon to practical purposes neutralization experiments were performed on a large number of rabbits with the following results:

It is found that there is a definite variation in the response of different rabbits to the skin preparatory effect of neutralized mixtures. The reactions obtained by injection of the same mixtures into different animals are divided into three categories: completely neutralized (CN) partially neutralized (PN) and non-neutralized (NN). It is also found that the potency of a given serum bears a mathematical ratio to all the types of reactions, namely to both the titer of serum giving complete neutralization in CN and PN rabbits and the number of rabbits belonging to *all the three types*. A scheme is proposed for carrying out accurate quantitative titrations of the protective value of serum (a complete report will appear elsewhere).

Normal and non-related heterologous sera have no neutralizing effect unless natural agglutinins are found for *B. typhosus*. Para A and B sera neutralize these factors in various dilutions. Whether the Para A and B antibodies are of a group character or identical with the *B. typhosus* antibodies remains to be determined.

9. An Improved Technique for the Comparison of Antiseptics by the Yeast Fermentation Method. SARA E. BRANHAM, Department of Bacteriology, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

A simple apparatus has been devised in which carbon dioxide production by fermenting yeasts can be measured with a fair degree of accuracy. By means of this apparatus the principles underlying the yeast fermentation method of comparing the antiseptic efficiency of silver and mercury compounds have been confirmed, put upon a quantitative basis, and shown to be applicable to many other types of disinfectants. In addition to giving a useful means of comparing antiseptics, this device offers an opportunity for the study of the phases of yeast fermentation under the influence of different compounds. The extreme simplicity of this technique commends it for general use.

10. A Comparison of the Hygienic Laboratory Test with the Method Used by the Department of Agriculture for Testing Disinfectants. C. M. BREWER AND GEORGE F. REDDISH, Food, Drug and Insecticide Administration, Department of Agriculture, Washington, D. C.

The advantages of the method for testing disinfectants used by the Department of Agriculture (described by Reddish in previous publications) as compared with the Hygienic Laboratory method are again set forth. Because of the very wide use of compounds chemically related to phenol for disinfecting purposes, members of this group were selected for comparing the two methods of testing. Twenty representative coal-tar disinfectants and 10 cresylic acid compounds were used, the *B. typhosus* phenol coefficient being obtained by both methods. The differences obtained by these methods are given. In some cases a higher phenol coefficient is obtained by the Department of Agriculture method and in others the Hygienic Laboratory method gives a higher figure, depending on the composition of the disinfectant. The figures show that the average percentage of variation between the two tests is too small to be of practical significance, seldom being higher than the limits of experimental error.

11. The Antiseptic Action of U. S. P. and N. F. Ointments. GEORGE F. REDDISH AND H. WALES, Food, Drug and Insecticide Administration, Department of Agriculture, Washington, D. C.

U. S. P. and N. F. ointments which are being used for antiseptic purposes were tested for bacteriostatic action by a method previously

described by the senior author. The ointments were streaked on the surface of plain nutrient agar (1.5 per cent agar) inoculated with *Staph. aureus*. Antiseptic action is indicated by a clear zone surrounding the streaked ointment, the remainder of the plate being turbid with the colonies of the test organism. Using this method of testing, it was found that only about one-half the U. S. P. and N. F. ointments so tested are in fact antiseptic.

12. The Variation of the Phenol Coefficients of Coal Tar Disinfectants with the Use of Different Test Organisms. BURTON G. PHILBRICK, Skinner, Sherman and Esselen, Inc., Boston, Mass.

For the purposes of this study, 4 samples of coal tar disinfectants having approximate *B. typhosus* phenol coefficients of 2/3, 5/6, 9/10 and 20, respectively, were selected as representative of that class of disinfectants.

The exact phenol coefficients of these samples were then determined by the modified Rideal-Walker method using *B. typhosus* as the test organism with and without the presence of organic matter. The *B. typhosus* phenol coefficient having been established, the phenol coefficients of these same samples were determined using as test organisms *Staph. aureus*, *B. diphtheriae*, *Strep. hemolyticus*, and *Pneumococcus*. In these determinations the culture medium was necessarily modified to suit the organism, but the modified Rideal-Walker method was adhered to as to temperature of medication, amount of inoculent, and time of exposure to disinfectant.

The phenol coefficients as determined are given in the following table:

SAMPLE	2/3	5/6	9/10	20
B. typhosus without organic matter	3.2	6.5	10.0	19.0
B. typhosus with organic matter	2.9	5.8	9.0	17.0
Staphylococcus aureus without organic matter	0.8	1.4	2.2	5.0
Staphylococcus aureus with organic matter	0.7	1.3	1.8	4.9
B. diphtheriae without organic matter	2.3	4.5	7.3	18.0
B. diphtheriae with organic matter	1.6	3.5	5.3	14.0
Streptococcus hemolyticus without organic matter	2.2	4.4	6.7	16.0
Streptococcus hemolyticus with organic matter	1.7	3.5	5.3	12.0
Pneumococcus without organic matter	3.3	6.6	10.0	23.5
Pneumococcus with organic matter	3.0	6.1	9.4	17.5

13. Bactericidal Effects of Sunlight as Affected by Glass. THEODORE W. HAUSMANN, Concordia College.

The relative rates of bacterial destruction by sunlight filtered through plate glass and through glass permeable to ultra-violet light (using plates $\frac{3}{16}$ inch in thickness) were determined with *Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus* (Reddish strain).

The first tests were made (August to October 15) by exposing pour plates and surface plates containing varying dilutions of each of these organisms to mid-day sunlight (1) without any intervening barrier, (2) through plate glass and (3) through glass permeable to ultra-violet light. (Vita-glass).

For these experiments special cubical boxes were constructed, 20 inches in diameter, with ventilating apertures to prevent condensation of moisture and to control possible differences in temperature (readings being made from 3 thermometers similarly placed in each box). The boxes were placed on adjustable supports to aid in keeping the glass covers and the contained Petri dishes at right angles to the sun's rays. Exposures varied from 30 minutes to 5 hours in the different experiments, varying with the known resistance of the organism and the variations in light intensity. After exposure, the plates were incubated for 2 days (37°C., except for *Serratia*, where 21°C. was used). The following conclusions may be made: (1) *Staphylococcus* was more rapidly affected by light than the other 2 organisms, one-half hour of exposure yielding marked contrasts; with *E. coli*, 1 hour was usually necessary, and even in August 2 hours were needed for marked contrasts with *Serratia marcescens*. (2) There were in all marked differences in colony size as well as in the number of colonies developing. (3) The ratio of the colonies developing may be roughly averaged down to the following proportions: 11, no sun or shade; 8, plate glass; 5, glass permeable to ultra-violet light and 3, direct sunlight.

The second set of tests (October and November) were made on the bacterial content of air in enclosed spaces. These tests offered greater difficulties, because of the frequency of air contaminants (chiefly molds), even when the exposures were made on the roof of a five-story building in the city. These air tests were, at first, made by washing the air exhausted for anaerobic jars in each box and left open during exposure. They were then closed and the exhausted air was washed through a series of water bottles, and the wash water used for plating. The plate-glass box air was higher in bacterial content than that in the other box covered

with glass permeable to ultra-violet light. Later, the boxes were treated with sulphur dioxide, ventilated (wet cotton filtering incoming air) and sprayed with bacterial cultures with atomizers. The air was pumped from these boxes into wash bottles as before, but as yet, practically no growth has been obtained, either because the wet spray reaches and adheres to the box surfaces or because the particles settle down during exposure to the sun.

14. The Use of Sodium Chloride in Culture Media for the Separation of Certain Gram-Positive Cocci from Gram-Negative Bacilli. JUSTINA H. HILL AND EDWIN C. WHITE, Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Md.

It was found that pH 6.0 sodium chloride agars, with salt concentrations from 2 through 20 per cent exerted marked inhibitory action on the growth of bacilli of the typhoid, paratyphoid, dysentery and colon groups, on *Proteus*, *Pseudomonas pyocyanea*, diphtheroids and *Bacillus anthracis*. The Gram-positive cocci studied tolerated high salt concentrations, all growing on transfer from 20 per cent sodium chloride agar.

In pH 6.0 broths with salt concentrations from 2 through 25 per cent, the same differential action was observed, although to a lesser degree than on agar.

With cultures of cocci and of bacilli, mixed in different proportions, the salt agars gave a selective growth of the cocci which could sometimes be recovered in pure culture. The use of 6, 8, 10 and 15 per cent salt agars greatly facilitated the isolation of Gram-positive cocci from mixed infections.

15. A Simplified Culture Medium for the Isolation of Neisseria gonorrhoeae. JAS. GIBBARD, Department of Pensions and National Health, Ottawa, Canada.

A new culture medium for the isolation and cultivation of *Neisseria gonorrhoeae* is described in detail. The basis of the medium is veal infusion, peptone, starch and agar plus human serum. The source of human serum is the surplus from specimens submitted for the Wassermann test. The medium is very easy to prepare and gives excellent growth of the gonococcus, usually within 15 to 24 hours. In the author's hands, it has proven superior to the media recommended by various workers within the past 10 years. It can be accurately standardized, thus assuring little if any variation between different batches.

16. An Analysis of Six Thousand Consecutive Routine Blood Cultures. W. G. HEEKS AND L. W. FAMULENER, St. Luke's Hospital, New York City.

In a series of six thousand consecutive blood cultures, (1,024) or 17.06 per cent, showed bacterial growth; of which 27 or 0.45 per cent of the total were considered as contaminants.

Seven hundred and seventy-one individuals yielded positive results, and in 253 instances, the blood cultures were repeated one or more times with positive findings.

The order of occurrence of various organisms appearing 15 or more times, in the series of positive individual cases, is as follows:

	Number of individuals	*Per cent of positive cultures*
Pneumococcus	214	27.7
B. typhosus	197	25.5
Strep. viridans	93	12.0
Strep. hemolyticus	89	11.5
Staph. aureus	45	5.8
Staph. aureus-hemolyticus	23	2.9
Staph. albus	15	1.9
Staph. albus-hemolyticus	15	1.9

It is of special interest to note the time periods at which the growth of various organisms appeared. The average time for *Streptococcus viridans* was 2.3 days, while *Streptococcus hemolyticus* appeared in 1.6 days; *B. typhosus* averaged 2.0 days; *Staphylococcus aureus* 2.0 days; *Staphylococcus albus* 1.5 days, and *Pneumococcus* (all types), 1.3 days.

17. Method of Securing Blood Serum from Convalescent Patients. F. M. MEADER, Department of Health, City of Detroit.

The value of blood serum from persons who have recovered from various communicable diseases, as a prophylactic agent is well substantiated in the medical literature. The problem of securing such serums in sufficient quantities has been solved by the Detroit Department of Health in the following manner:

The Department decided to secure serum from convalescent cases of measles, for distribution to institutions and doctors wishing to protect young or feeble contacts. The name and address of adults who had measles during the past year was noted. A form letter was sent to each one, offering ten dollars for a sample of blood. A nurse was detailed to arrange with donors who accepted the offer, to come to the laboratory where a doctor would secure the blood. By this means 2,200 doses were

secured. Details regarding method of handling the serum, amount of blood obtained, the cost, and distribution, are given in the paper.

The same method has been used to secure blood serum from persons convalescent from scarlet fever, whooping cough, mumps, chickenpox and poliomyelitis, with as good results. The serum section of our laboratory promises to be a very important service in our Health Department.

18. Seasonal Variation in Antitoxin Content of Human Blood. ROGER G. PERKINS, RALPH HEEREN, EMERSON MEGRAIL, AND A. B. GROSSMAN, Department of Hygiene and Bacteriology, School of Medicine, Western Reserve University, Cleveland, Ohio.

A 12-month study of changes in the antitoxin content of the blood in 2 age groups involving 30 individuals, with consideration of possible relations to the seasonal incidence of diphtheria. This paper forms part of a series of studies of seasonal and periodic variations in a group of diseases.

19. Further Studies on the Immunology of the Pneumococcus. H. M. POWELL, G. H. A. CLOWES, AND W. A. JAMIESON, Eli Lilly and Company, Indianapolis, Indiana.

Further work upon the immunology of the pneumococcus has shown that toxic filtrates of this organism can be reliably evaluated by skin tests on different species and immune serums can be prepared which are neutralizing. Such pneumococcus serums can be concentrated. It has been found that standardization of pneumococcus filtrates and serums by mouse lung tests as previously reported in Proceedings of the Society for Experimental Biology and Medicine, 1926, vol. **23**, pp. 331–337, is more or less uncertain and difficult to control. The very high degree of purification of serums reported has been impossible to obtain as judged by skin neutralization tests. The skin test method, however, offers a means by which pneumococcus toxic filtrates and serums can be measured.

20. An Auxiliary Antigen for the Kahn Test. R. L. KAHN, G. LUBIN AND E. MCDERMOTT, Michigan Department of Health, Lansing, Mich. and University of Michigan Hospital, Ann Arbor, Mich.

Several years ago one of us described an antigen for use in the "presumptive" Kahn procedure. That antigen, while more sensitive than standard antigen, lacked the property of uniformity in sensitiveness due

to our meager knowledge of the principles of antigen standardization. With a better understanding of these principles, it was possible to prepare and standardize an antigen more sensitive than standard antigen and possessing at the same time a high degree of uniformity. This antigen was named "sensitized" antigen.

Three reagents are necessary in the preparation of sensitized antigen: (1) standard antigen, (2) sensitizing reagent which consists of an alcoholic solution of dried ether extractives obtained from the preparation of standard antigen and (3) an alcoholic solution containing 6 mgm. cholesterol per cc. Sensitizing reagent added to standard antigen up to certain limits, increases the sensitiveness of the final product. When adding cholesterolized alcohol to this product, there is a further tendency for increasing the sensitiveness. By the use of sensitizing reagent and cholesterolized alcohol, standard antigen can thus be increased in sensitiveness to the level of sensitized antigen, by the employment of standard sensitized antigen as a control.

The presumptive procedure with sensitized antigen is markedly more sensitive than the 3-tube Kahn test in treated cases, this increase being as high as 30 per cent. In diagnostic cases the increase in sensitiveness of the presumptive over the regular test depends on the type of syphilis among the patients tested. At the University of Michigan Hospital this increase approximates 2 per cent. With regard to the specificity of the presumptive procedure, no opportunity has presented itself thus far for careful clinical studies. Preliminary studies indicate however that this procedure is specific to a high degree.

21. Studies on the Kahn Reaction with Spinal Fluids. R. L. KAHN, E. MCDERMOTT AND G. LUBIN, Michigan Department of Health, Lansing, Mich. and University of Michigan Hospital, Ann Arbor, Mich.

The original Kahn test with spinal fluids was highly specific and conservative in sensitiveness. Special precautions leading to conservative sensitiveness were considered essential because the method of precipitating the globulin of spinal fluid with saturated ammonium sulfate resulted in the presence of about 3 per cent of this salt in the final globulin solution—a concentration which, even in the absence of positive serum or spinal fluid, is sufficient to yield slight precipitates with antigen suspension when the latter is prepared at the usual serum titer. To overcome this difficulty the lipid particles in the antigen suspension were made more soluble by preparing the suspension with a

larger amount of saline than that indicated by the antigen titer for serum tests. Furthermore, only 40 instead of 50 per cent of the saturated ammonium sulfate was used in the separation of the globulins. Both of these precautions tended to reduce the sensitiveness of the test.

It was recently observed that by employing small (7.5 by 1 cm.) instead of large test tubes for mixing the spinal fluid with the ammonium sulfate, and by using a few simple precautions in manipulation, it is possible to remove almost completely the supernatant fluid from the globulin precipitates, thus reducing to a minimum the amount of sulfate in the globulin solution. This technique made practicable a 50 instead of 40 per cent saturation of the spinal fluid with ammonium sulfate, yielding all of the fluid globulins instead of only a fraction of the globulins. It also made practicable the use of an antigen titer similar to that employed in serum tests. In a series of 400 spinal fluid tests run under these conditions, some increase in sensitiveness was observed without encountering false positive reactions.

It was also found that sensitized antigen may be used in the spinal fluid procedure. The reactions obtained in a group of 300 spinal fluids indicate a higher degree of sensitiveness with freedom from false reactions. A still more sensitive spinal fluid reaction is obtained with sensitized antigen when the globulin concentration of these fluids is increased 20 instead of 10 times the concentration in the original fluid. The group of fluids studied is as yet too small to establish the specificity of this method but indications are that this procedure is also specific.

It appears that the Kahn reaction lends itself to the employment of spinal fluid procedures of marked sensitiveness and specificity in the detection of neurosyphilis.

22. Primary Diphtheric Vaginitis in Children. ANNA I. VAN SAUN AND DOROTHY V. FREEMAN, Board of Health, City of Paterson, N. J.

A short resumé is given of the few authentic cases of primary diphtheric vaginitis found in the literature.

The authors report a case of apparently primary diphtheric vaginitis in a child 8 years of age, giving a description of the clinical aspects of the case and the bacteriologic findings.

Cultures taken from the throat of the patient contained no diphtheria bacilli. Those from the nose contained a diphtheria-like bacillus which, on being tested for virulence, gave negative results. Cultures from the vagina contained large numbers of typical diphtheria bacilli

which gave a positive virulence test. The case is thought to have been probably primarily a vaginal infection since nose and throat cultures were consistently negative.

Attention is called to the necessity for release cultures in cases of vaginitis as well as in other forms of diphtheria. The necessity for virulence tests is also stressed when the determination of the original avenue of infection is indicated.

23. Corynebacterium ulcerans: Its Possible Epidemiological Importance. RUTH GILBERT, Division of Laboratories and Research, New York State Department of Health, Albany, New York.

The close resemblance of *Corynebacterium ulcerans* in young cultures to the diphtheria bacillus and its coccoid appearance after longer incubation makes animal inoculation necessary for identification. Since most throat cultures receive a morphological examination only, the prevalence of infections due to *C. ulcerans* may be greater than past experience has indicated. It has proved to be the incitant in at least two epidemics in widely separated districts in New York State.

24. The Influence of Carbon Dioxide on Diphtheria Toxin Production and Preservation. WAYNE N. PLASTRIDGE AND LEO F. RETTGER, Laboratory of General Bacteriology, Yale University, New Haven, Conn.

In the study of the influence of varying amounts of CO_2 on the diphtheria bacillus and its extracellular toxin both the subcutaneous and intracutaneous methods of toxicity determination were employed. The carbon dioxide and oxygen were supplied to the cultures by an aeration system with which fairly constant amounts of these gases could be maintained.

Aeration of cultures with CO_2-free atmospheres containing from 10 to 50 per cent oxygen resulted in marked irregularity in growth and in the toxin content of *C. diphtheriae* cultures, and permitted an increased rate of destruction of toxin once it was formed. The rate of destruction of toxin in these cultures was directly proportional to the concentration of oxygen in the atmospheres passed over the cultures.

The presence of from 3 to 10 per cent CO_2 in the atmospheres passed over *C. diphtheriae* cultures resulted in increased growth and toxin production. Uniform maximum toxin formation with a given medium was obtained in cultures grown under an atmosphere containing 10 per

cent CO_2 and from 10 to 20 per cent oxygen. No decrease in toxicity occurred in any of these cultures on prolonged incubation.

Growth and toxin production were slightly retarded in cultures aerated with an atmosphere containing 5 per cent oxygen and markedly retarded in cultures aerated with an atmosphere containing only 1.5 per cent oxygen.

Tests conducted for the purpose of determining the mechanism responsible for the preservation of diphtheria toxin in cultures grown under an atmosphere containing from 3 to 10 per cent CO_2 showed the following: (1) *In the absence of added* CO_2, the rate of destruction of toxin by molecular oxygen, within the limits of pH 7.0 to pH 9.0, is inversely proportional to the hydrogen ion concentration of the toxin solution; (2) *In the presence of added* CO_2, the destruction of diphtheria toxin by molecular oxygen is largely or entirely prevented.

Toxic culture filtrates were adjusted to different hydrogen ion concentrations and stored in sealed tubes under nitrogen, CO_2, air, and in *vacuo*. Only the filtrates stored under CO_2 remained highly toxic after 180 days storage at 37°C.

The general conclusion may be drawn from the data presented, that CO_2 plays an important rôle in the growth and toxin production of *C. diphtheriae*, mainly by (1) stimulating growth and toxin formation, (2) controlling the reaction of the culture medium during the growth of *C. diphtheriae*, and (3) preventing the destruction of the toxin once it is formed.

25. Bacteriological Characteristics of Streptococcus epidemicus Isolated During a Septic Sore Throat Epidemic. ELLIOTT S. ROBINSON, Antitoxin and Vaccine Laboratory, Jamaica Plain, Boston, Mass.

The morphological and cultural characteristics of strains of *S. epidemicus* isolated from patients affected during an extensive epidemic of septic sore throat and from the cow whose milk was the probable source of the infection were studied. The strains isolated were alike in being encapsulated hemolytic streptococci growing as moist colonies on milk-blood-agar medium; in being virulent for mice; in producing acid in glucose, lactose, sucrose, and salicin media but not in inulin, mannite, or raffinose; and in being low acid producers (pH 5.3 to 5.7) in glucose broth. In short, the organisms studied were entirely similar to those described by others from previous epidemics of septic sore throat. The course of the natural infection in the cow was also followed for a time.

26. The Origin and Nature of the Epithelial Cell Inclusions in Trachoma and Their Relationship to the Disease. IDA A. BENGSTON, Hygienic Laboratory, U. S. Public Health Service, Washington, D. C.

A microscopical study has been made of film preparations from numerous cases of trachoma with a view to determining the nature of the inclusion body and its relationship to the disease. It appears that the inclusion body originates from a rod-shaped microörganism and that the "elementary bodies" of Lindner are modified forms of the rod-shaped microörganism. It is probable that the lytic substances present in the conjunctival fluids and in the conjunctival cells act on the rod-shaped bacteria reducing them to the small, indefinite pleomorphic forms in which they occur outside the cells and that the inclusion bodies in the epithelial cells are formed as the result of the action of lytic or digestive substances on a group or colony of bacteria contained within the cytoplasm of the cell. Forms resembling the "elementary bodies" and the bipolarly stained "free initial bodies" of Lindner have been produced experimentally by inoculating the conjunctiva of guinea pigs with cultures of certain Gram-negative rod-shaped microörganisms isolated from cases of human trachoma. The study tends to confirm the work of Noguchi who found a certain Gram-negative rod associated with the disease and to disprove the theory of Lindner that the inclusion body originates from an organism which is non-bacterial in nature.

27. Epizootic Lymphadenitis in the Guinea-Pig. EMERSON MEGRAIL AND ROBERT N. HOYT, Department of Hygiene and Bacteriology, Western Reserve School of Medicine, Cleveland, Ohio.

Thirty-five guinea-pigs were examined in an outbreak of epizootic lymphadenitis, commonly known as "lumps." Either a hemolytic or non-hemolytic streptococcus and, in addition, *Staphylococcus albus* were isolated from each animal. Experimentally, both types of the streptococcus are able to produce the disease and the staphylococcus is apparently a secondary invader. Transfer experiments through skin abrasions were successful.

28. Experimental Transmission of Epizootic Encephalitis of Foxes. R. G. GREEN AND N. R. ZIEGLER, Department of Bacteriology and Immunology, University of Minnesota, AND E. T. DEWEY AND J. E. SHILLINGER, Bureau of Biological Survey, Washington, D. C.

Epizootic encephalitis of foxes has been transmitted to well, healthy

foxes by the injection of brain and spinal cord virus obtained from animals dying of the natural disease. The identity of the disease with the artificial infection is shown by the period of infection, the symptoms and pathology produced. The disease has been transmitted by intracranial, cisterna and intramuscular injection. The infection has been transmitted in series from one experimental animal to another.

29. *Experimental Tularemia Infection in Birds.* R. G. GREEN AND E. M. WADE, Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minnesota, and the Division of Preventable Diseases, Minnesota State Board of Health.

Skin inoculation of the ruffed grouse and the Hungarian partridge with material infective for tularemia, results in a fatal infection, terminating in about 7 days. Upon intramuscular injection a generalized infection usually occurs in the common pigeon. The infection does not result fatally and no symptoms of the disease are evident. *Bact. tularense* appears to be eliminated from the body of the pigeon in about 7 days.

Intramuscular injection of infective material in the ring-necked pheasant results in a generalized invasion with no apparent symptoms of the disease. Recovery from the infection occurs after about 7 days.

30. *Chromogenesis in B. salmonicida.* DAVID L. BELDING AND JULIA G. ARROWOOD, Boston University School of Medicine and Evans Memorial Hospital, Boston, Mass.

B. salmonicida, the causative agent of fish "Furunculosis," produces a diffusible brown pigment when grown upon favorable media at room temperature. The pigment is slightly soluble in alcohol, but not in chloroform or ether. Pigment formation may be temporarily inhibited by cultivation under anaerobic conditions, upon acid media, upon media containing certain antiseptics, and at a temperature above 30°C. Chromogenesis is restored when the organism is subcultured under favorable conditions.

A sport strain obtained from a 7-year-old stock culture proved non-chromogenic. Except for pigment formation it was bacteriologically and serologically identical with the chromogenic strain from which it was derived and with strains of *B. salmonicida* from other sources. Efforts to convert the non-chromogenic into a chromogenic strain by various methods of cultivation have so far been unsuccessful.

31. A Preliminary Note on the Cytology of Mycobacterium tuberculosis. GEORGES KNAYSI, Department of Dairy Industry, Cornell University, Ithaca, N. Y.

The young cell of *Mycobacterium tuberculosis* consists of a very thin membrane presenting thickened areas and granular appendages over its internal surface. The cytoplasm is made up of dense, chromatic material and many vacuoles. In the chromatic material are granules of a hyperchromatic substance apparently identical with the substance of the membrane. Those granules vary in size and number. The larger ones are most commonly two in number, one near each pole of the cell. A third granule may be in the middle, or two or more distributed along the cell. Almost invariably, a granule lies contiguous to a vacuole. The substance making up the granules and the membrane is highly elastic and is extremely resistant to chemicals. It was not dissolved, at the end of one week, by glacial acetic acid, 5 per cent sulfuric acid, 5 per cent sodium hydroxide, or chloroform. It takes up strongly iodine, all of the basic dyes tried, and the fat dyes. In taking up dyes, granules and membrane swell. Especially with fat dyes is this effect remarkable when both the membrane and the granules may attain more than twice their normal thickness. This substance is the principal cause of the acid and gram resistance of the organism. The granules, which are very conspicuous in an actively growing culture, must be, because of their great adsorptive power, the foci of intense chemical activities, and they probably play a preponderant rôle in the nutrition of the cell. Two of these granules are usually formed, when the cell is dividing at the contiguous poles of the daughter cells, but their origin and their behavior, as far as are known, do not justify considering them as nuclei as we know nuclei in higher plants and animals.

No plasmodesma were observed between cells, and no wax, neutral fat, or free fatty acids were demonstrated microchemically either within the cell or around it, as is generally believed, in cultures from 5 to 24 hours old.

32. Filtration Experiments with the Granular Form of the Tubercle Bacillus. RALPH R. MELLON AND ELIZABETH L. JOST, Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa.

For the past 5 years French workers especially have been most active in their attempts to demonstrate a filterable phase of the tubercle bacillus. Despite their asservations that their contention is proved, the evidence submitted has been quite inadequate in almost every instance.

Progressive disease has not been produced, and they have not been able to isolate the tubercle bacillus from supposed (?) lesions where acid fast bacilli were demonstrable in the smears. Moreover, histological evidence for tubercle has usually been lacking.

In 1926 we reported in a preliminary way definitely positive results, satisfying all the above criteria. But the inoculation conditions, as well as the material itself, were somewhat anomalous and it was desired to repeat the experiment with other similar material and under varying conditions of inoculation.

Our inoculation results have been categorically positive *only* when the inoculation material contained large numbers of what appeared to be acid fast granules. Out of a total of 37 animals, 4 have yielded widespread progressive lesions of the disease. From 2 of these animals the tubercle bacillus in pure culture has been recovered. These 4 positive animals are the result of inoculation of granular material from 2 sources; one, a sputum; the other, a pulmonary miliary tuberculosis.

Although many of the remainder of the 37 animals had enlarged lymph glands with acid fast bacilli, thus corresponding with the bulk of the French work, we do not consider such evidence adequate for the filtrability contention unless supported by the evidence shown for our 4 definitely positive animals.

83. Permeability of the Respiratory Tract to Old Tuberculin. PAUL F. CLARK AND (by invitation) WILLIAM S. MIDDLETON AND OTIS M. WILSON, University of Wisconsin, Madison, Wisconsin.

Several acute attacks of asthma occurred in a student whose duties exposed him to the vapor of tuberculin in a room where this material was being cleared by means of a Sharpless centrifuge. As the patient gave a negative von Pirquet reaction, we wondered whether so poor an antigen as tuberculin could possibly have been the means of sensitizing this individual through the respiratory tract. We have succeeded in demonstrating that it is easy to sensitize guinea-pigs to such excellent complex antigens as horse serum and such simple proteins as crystallized egg albumen or excelsin by the use of a fine spray in a closed chamber. We have also sensitized guinea-pigs to old tuberculin by this method so that subsequent intravenous injections of minute amounts have resulted in fatal anaphylactic shock.

84. A Study of Bacillus Calmette-Guerin (B.C.G.). 1. Biological Characteristics, Cultural "Dissociation" and Animal Experimenta-

tion. S. A. PETROFF, ARNOLD BRANCH AND WILLIAM STEENKEN, JR., Research and Clinical Laboratory, Trudeau Sanatorium, Trudeau, N. Y.

Prophylactic immunization against tuberculosis has been extensively carried on in Europe. The method consists of feeding infants with living avirulent cultures of tubercle bacilli known as *Bacillus Calmette-Guerin* (B.C.G.). The claims have been that the organism does not produce progressive tuberculosis and that it possesses remarkable antigenic properties for rendering humans and animals immune to tuberculosis. Our studies since 1925 can be summarized as follows:

1. The organism when cultivated on a medium advocated by the sponsors of this method, only on very rare occasions produces tuberculosis in animals terminating in death.

2. After the organism has been dissociated into "R" and "S" colonies, it was found that the "R" colony produces tubercles and the lesions have a tendency to heal, while the "S" invariably produces extensive lesions leading to the death of the animal.

3. The immunity established in guinea pigs with this organism is not greater than that obtained when animals are injected with heat-killed tubercle bacilli.

The use of this type of vaccination is not entirely free from the danger of infection in human beings. More work must be done on a larger scale, especially in cattle, in order to substantiate or refute the claims of the authors of this method.

35. The Pathogenicity of the Species of the Genus Brucella for Monkeys. I. FOREST HUDDLESON, Department of Bacteriology, Michigan State College, East Lansing, Mich.

Experiments to determine the pathogenicity of strains of each of the 3 species of the genus *Brucella* for monkeys by the oral route reveal that the porcine species is the most pathogenic. This is true of this particular species regardless of the source of isolation, (hog, cow, or man).

The true bovine abortus species, regardless of its source, will produce a mild form of the disease followed by rapid recovery after the ingestion of a massive dose or repeated small doses over a considerable period of time.

Small doses of a recently isolated strain of the melitensis species will produce the disease in monkeys, but old cultures fed daily for a long period of time fail to produce the disease.

In nearly every case one agar slant of the porcine species has produced the disease in 10 to 15 days without recovery.

These findings are suggestive of the difference in virulence of the species for man.

36. The Significance of Brucella abortus Agglutinins in Human Sera. C. M. CARPENTER, RUTH BOAK, Diagnostic Laboratory, New York State Veterinary College, Cornell University, Ithaca, N. Y., AND O. D. CHAPMAN, Department of Bacteriology, School of Medicine, Syracuse University, Syracuse, N. Y.

A comparison of the results obtained by investigators in various states of the percentages of Wassermann serum samples showing *Br. abortus* agglutinins is reported. A study of 4,050 serum samples collected at Syracuse, N. Y. showed 7.3 per cent to contain *Br. abortus* agglutinins while 2.4 per cent of 955 samples collected from Bellevue Hospital, New York City, were positive.

The blood serum from individuals fed pasteurized milk with a high *Br. abortus* agglutinin titre showed no evidence of the absorption of the antibodies. Examinations of the blood serum of individuals having recovered from undulant fever demonstrated the antibodies to be present in some cases for a period of 2 years after the symptoms had subsided. *Br. abortus* has been isolated from the blood of patients with undulant fever showing no agglutinins as well as from cases with very few agglutinins in their serum. Because of such findings it is impossible to designate the minimum serum titre diagnostic of undulant fever.

37. The Respiration of the Abortus-Melitensis Group. M. H. SOULE, Hygienic Laboratory, University of Michigan, Ann Arbor, Mich.

The respiratory quotients were determined for human, porcine and bovine cultures of *Brucella abortus* and for cultures of *B. melitensis*. The organisms were grown on agar slants in *h*-tubes connected to manometers. Samples of the supernatant gases were removed from the respiratory chambers and analyzed with the modified Henderson-Haldane burette. The CO_2 held in the media (dissolved and fixed) was determined by aeration. From the total amount of O_2 consumed and CO_2 produced the corrected real respiratory quotients were calculated. The rates of growth were followed manometrically. The observed manometric readings were compared with those computed from the analyses of the gases and thus served as a check on the analyses. Manometrically the porcine strains gave the most rapid growth and the

melitensis strains the slowest growth on all of the mediums investigated The human strains according to their rate of growth were either of the porcine or bovine type.

On plain infusion agar there were no marked differences in the corrected real respiratory quotients for the various strains, indicating similar metabolic activities. The average corrected real respiratory quotient was 1.056, much higher than the accepted value of 0.81 for protein substances. This value may be taken as correct for the experimental conditions which prevailed; but it probably does not represent the actual intracellular respiratory change. Secondary extracellular changes such as carboxylase action, where CO_2 is formed, could increase the total yield of CO_2 with a corresponding increase in the respiratory quotient.

The addition of 2 per cent glucose to the medium favored the growth of all of the cultures, and exerted a sparing action on the decomposition of the proteins implying a utilization of glucose. There was a distinct decrease in the quantities of fixed CO_2 in these experiments, the bovine strains gave the smallest amounts. The average corrected real respiratory quotient on the glucose containing medium was 1.046.

On 5 per cent glycerol agar rich growths were obtained. The average corrected real respiratory quotient was 0.950, distinctly lower than on the other two mediums but much higher than the theoretical value of 0.857 for the combustion of glycerol. The smallest amounts of fixed CO_2 observed were obtained with this medium indicating the utilization of glycerol for growth energy with a minimum amount of protein decomposition.

Distilled water suspensions of the organisms were killed when spread over the surface of beef extract agar in Kitasato plates and subsequently aerated with CO_2-free air, CO_2-free tank nitrogen ($\pm$ 5 per cent O_2) or purified tank N_2 (CO_2-free, oxygen free) at the rate of 100 cc. per min. Luxuriant growths were obtained if a small amount of CO_2 was added to the aerating gas. These experiments are being continued in an effort to detect some variation in the gaseous metabolism of these strains.

38. An Organism Isolated from Three Cases of Bacteremia After Appendicitis. EMIL WEISS, Department of Bacteriology, Loyola University School of Medicine, Chicago, Ill.

The isolated organism shows the following properties: short rods with round ends, 0.3 to 0.5 micron wide, 1 to 1.5 microns long, occurring singly, sometimes in pairs. The organism is non-motile, has no flagella,

does not form spores, has no capsule and is Gram-negative. The colonies on gelatin are small, circular and transparent; liquefaction occurs on gelatin stab. The colonies on agar are small, transparent and glistening. The broth shows a slight turbidity, has no pellicle and forms a scant sediment. Litmus milk remains unchanged after several days of incubation. On potato only a scant growth could be obtained. The organism does not produce indol, and does not reduce nitrates. It does not form acid or gas in carbohydrate media. It produces no characteristic odor and is a facultative anaerobe. The optimal temperature for growth is 37°C. It is non-pathogenic for rabbits, guinea pigs, white rats and mice. According to the described properties this organism must be classified in the order Eubacteriales, and genus Alcaligenes. Within this genus it seems to be principally related to the *Alcaligenes abortus* (Bang).

39. The Application of Weiss' Flagella Method for the Staining of Spirochetes. EMIL WEISS, Department of Bacteriology, Loyola University School of Medicine, Chicago, Ill.

The above method gives the following advantages, if applied to the demonstration of spirochetes: the mordant keeps, and does not need to be freshly prepared; the spirochetes appear large and deeply stained; no sediment is noticeable; and when using a combination of a contrasting basic and an acidic dye, the bacteria take the basic dye, the spirochetes the acid dye.